Experientia Volume 51 / 1995 Pages A1–A101 Basel, February 1995

27th Annual Meeting of the Swiss Societies for Experimental Biology (USGEB / USSBE)

March 30 / 31, 1995
University of Fribourg
Fribourg

Contents

Page	Abstract	Section
A1	(S01-01/S01-14)	**Oxygen sensitivity**
A3	(S02-01/S02-10)	**Neurobiology of learning**
A5	(S03-01/S03-36)	**Modelling and therapy of genetic disorders**
A11	(S04-01/S04-41)	**Signal transduction in plants and plant biology**
A18	(S05-01/S05-65)	**Chromosome structure and regulation of gene expression**
A29	(S06-01/S06-65)	**Signal transduction in animal cells**
A40	(S07-01/S07-41)	**Developmental neurobiology**
A47	(S08-01/S08-10)	**Diabetes susceptibility genes**
A49	(S09-01/S09-07)	**Scanning probe microscopes in biology**
A50	(S10-01/S10-15)	**Membrane biogenesis**
A53	(S11-01/S11-28)	**Calcium-binding proteins**
A58	(S12-01/S12-24)	***Drosophila* genes and neurogenesis**
A62	(S13-01/S13-22)	**Proteases in cell-cell and cell-matrix interactions**
A66	(S14-01/S14-35)	**Structure-function relationship in membrane proteins**
A72	(S15-01/S15-05)	**Quality assessment of animals in experiments**
A73	(S16-01/S16-43)	**Medical biochemistry, physiology and toxicology**
A80	(S17-01/S17-28)	**Synaptic transmission and molecular neurobiology**
A85	(S18-01/S18-08)	**Molecular biology of parasites**
A86	(S19-01/S19-08)	**RNA processing and translation**
A88	(S20-01/S20-19)	**Cell cycle and apoptosis**
A91	(S21-01/S21-18)	**Cellular and molecular biology of epithelial transport**

A95 **Authors Index**

Oxygen sensitivity

S01-01

HYPOXIA, A NOVEL INDUCER OF ACUTE PHASE GENE EXPRESSION IN HUMAN HEPATOMA CELL LINES

Wenger R.H., Rolfs A., Marti H.H., Bauer C. and Gassmann M.
Institute of Physiology, University of Zürich-Irchel, Winterthurerstrasse 190, CH-8057 Zürich. Fax: 01 364 05 64

Human hepatoma cell lines such as HepG2 and Hep3B are established models to study hypoxic induction of erythropoietin. Using a differential screening approach, we identified additional hypoxia-regulated genes, including aldolase and α_1-antitrypsin, a member of the acute phase (AP) responsive protein family. AP proteins are liver-derived plasma proteins whose production during inflammation is either upregulated (positive AP reactants) or downregulated (negative AP reactants) by proinflammatory cytokines. HepG2 and Hep3B cells are known to respond to cytokines *in vitro* by a 2 to 10 fold induction of positive AP reactants. We showed that hypoxia similarly led to: (i) a 3 to 7 fold induction of steady-state mRNA levels of the positive AP reactants α_1-antitrypsin, α_1-antichymotrypsin, complement component C3, α_1-acid glycoprotein, haptoglobin and hemopexin, (ii) a downregulation of the negative AP reactant albumin, (iii) an upregulation of the negative AP reactant transferrin, and (iv) unchanged mRNA levels of the positive AP reactants α- and β-fibrinogen. These findings suggest that the adaptive response to hypoxia overlaps with, but is not identical to the AP response mediated by proinflammatory cytokines in hepatoma cells.

S01-02

ISOLATION AND CHARACTERIZATION OF A NOVEL ZINC- AND HAEM-CONTAINING OXYGEN-BINDING PROTEIN FROM BOVINE BRAIN

Hasler, D.W., Fundel, S.M., Bogumil, R., Pountney, D.L. & Vašák, M. Biochemistry Institute, University of Zürich, CH-8057 Zürich.

We have isolated a cytosolic haem- and zinc-containing protein from bovine brain which binds oxygen with high affinity. Gel permeation, SDS-PAGE and mass spectral analysis reveal a globular, monomeric protein with a mass of 31.2 kDa. The partial polypeptide sequence (*ca.* 20 % of the total amino acids) determined on four large peptide fragments does not correspond to any known protein. Spectroscopic studies, i.e. UV-vis, MCD and EPR, indicate a type b haem with a histidine proximal ligand and the sixth coordination site occupied either by O_2 in the native Fe(II) form or OH^-/H_2O after oxidation to the Fe(III) form. The haem group readily forms complexes with the endogenous ligands CN^-, CO and NO, the spectral features of which are similar to those of the corresponding haemoglobin and/or myoglobin derivatives. By analogy with the giant haemoglobins from oligochaete, the coordinated Zn atom may modulate O_2-binding.

S01-03

THE MOLE RAT SHOWS STRUCTURAL ADAPTATION TO HYPOXIA IN LUNG AND MUSCLE TISSUE.

H.R. Widmer, E.R. Weibel, H. Claassen, L. Tüscher, C.R. Taylor+ and H. Hoppeler; Institute of Anatomy, University of Berne, CH-3012 Berne and +Harvard University, Cambridge, MA 02138, U.S.A.

The metabolism of the subterranean mole rat (Spalax ehrenbergi) was found to be relatively unaffected by hypoxic-hypercapnic conditions, therefore showing functional adaptations to fossorial life. In the present study we investigated whether mole rat lung and muscle tissue would show structural modifications favoring oxygen transfer in hypoxia. To this end we compared mole rats to weight-matched white rats. It was observed that muscle mass in mole rats was significantly smaller. Mole rats do have a significantly bigger muscle mass-specific lung volume with a significantly greater muscle mass-specific capillary surface area and alveolar surface area (by 70%). In addition, oxygen diffusing capacity into erythrocytes, membrane oxygen diffusing capacity and pulmonary oxygen diffusion capacity were all increased compared to white rats (by 40%). Volume density of mitochondria in muscle fibers of mole rats was found to be significantly greater (by 45%). Capillary length per muscle fiber tended to be bigger (by 30%). Similar results were obtained for deltoideus muscle with a significantly greater number of capillaries per muscle fibers. We conclude from our results, that several morphological parameters facilitate oxygen transport into tissue compartments, thus enabling mole rats to be highly active at low oxygen concentrations.

S01-04

STEP vs. PROGRESSIVE EXERCISE : THE KINETICS OF CREATINE PHOSPHATE HYDROLYSIS IN HUMAN MUSCLE.

P. Cerretelli, T. Binzoni, E. Hiltbrand and T. Yano. Depts of Physiology and Radiology, Univ. of Geneva (Switzerland) and CIGABIN, Univ. of Milano (Italy).

The kinetics of readjustment of the muscle oxidative machinery in man upon step work load ($\dot{w}$) changes has been assessed so far from measurements of gas exchange kinetics at the lung level. It was shown that, compared to rest, for given $\Delta\dot{w}$, priming exercise may elicit faster $\dot{V}O_2$ readjustment rates (1). However, it has not been established whether the above change is a result of a faster kinetics of the oxidative reactions or, alternatively, of the reduction of different exoergonic mechanisms, e.g. anaerobic glycolysis or of a change of the tissue O_2 stores. The problem could be solved by assessing directly by ^{31}P-NMRS the kinetics of phosphocreatine (PCr) hydrolysis at the muscle level (2). Nine normal subjects carried out in a 90 cm bore modified (SMIS, UK) Picker system (1.5T), series of contractions by the plantar flexors reaching given pre-set submaximal loads either in a single step or progressively. Should priming exercise influence the rate of TCA cycling and/or of oxidative phosphorylation, the PCr concentration at the target loads should be different in the two cases reflecting different energy deficits. The results show that the rate of readjustment of oxidations at the muscle level is not significantly affected by priming exercise. (1) di Prampero P.E. et al., J. Appl. Physiol., 66: 2023-2031 1989. (2) Binzoni T., et al. J. Appl. Physiol., 73: 1644-1649, 1992.

S01-05

HYPOXIA-INDUCIBLE EXPRESSION OF THE ERYTHROPOIETIN GENE IN HUMAN, MONKEY AND MURINE BRAIN

Marti HH, Wenger RH, Rivas LA, Straumann U, Bauer C, Gassmann M
Physiologisches Institut, Universität Zürich-Irchel, Winterthurerstr. 190, CH-8057 Zürich, Fax: 01/ 364 05 64

Erythropoietin (EPO) is the primary regulator of mammalian erythropoiesis. Production of EPO by the kidney and the liver is upregulated in response to hypoxia. We and others have recently shown EPO and EPO-receptor (EPO-R) gene expression in rodent brain and in human tumors of the central nervous system. These findings raised the question as to whether EPO has other functions beside its role in erythropoiesis. The aim of this study was to investigate if EPO gene expression is a general phenomenon in the brain of mammals and, if so, to analyse its oxygen-dependent regulation. Using reverse transcriptase mediated-PCR we indeed detected EPO mRNA in biopses from human hippocampus, amygdala and temporal cortex and in all tested brain areas of monkey (maccaca mulatta): cortex, cerebellum, hippocampus, hypothalamus and nucleus caudatus. As in kidney and liver, EPO gene expression was found to be hypoxia-inducible in various areas of monkey brain. In mouse, EPO mRNA was detected in isolated primary astrocytes and was induced after incubation at 1% oxygen compared to normoxic controls. Finally, we showed that EPO-R mRNA is also present in the brain of man and monkey. These findings suggest that EPO and its receptor have a new, yet to define role in the mammalian brain.

S01-06

EMBRYOID BODIES AS AN *IN VITRO* MODEL OF EARLY ERYTHROPOIESIS

Bichet S., Marti H.H., Baier-Kusterman W., Bauer C. and Gassmann M.
Physiologisches Institut, Universität Zürich, Winterthurerstr. 190, 8057 Zürich. Fax: 01/364 05 64.

Erythropoiesis during the very early stage of murine development can be analysed in an *in vitro* model based on differentiated embryonic stem (ES) cells. These blastocyst-derived cells are able to differentiate in culture to generate so called embryoid bodies (EBs) forming endoderm, mesoderm and ectoderm, thus partially recapitulating several aspects of embryogenesis. We adapted a DAF-based (2,7-diaminofluorene) staining method to detect the pseudoperoxidase activity of hemoglobin *in situ*. EBs containing DAF-positive cells were observed as early as differentiation day 5-6. To define whether erythropoietin (EPO), the main regulator of erythropoiesis, is regulated in an oxygen-dependent manner in EBs as it is in fetal liver and adult kidney, we established a competitive RT-PCR. This quantitative assay showed elevated EPO mRNA levels after exposure to low oxygen (1%) compared to the normoxic control. In addition, RT-PCR analysis of total RNA also revealed the presence of EPO-receptor RNA in EBs. At present, we are testing the possibility of measuring the hemoglobin content of EBs grown under normoxic and hypoxic conditions by DAF-mediated colorimetry. Furthermore, we expect to define the EPO-producing and the EPO-receptor-containing cells in EBs by immunofluorescence using tissue-specific markers.

S01-07

PUTATIVE ROLE OF AP-1 BINDING FACTORS IN GEL MOBILITY SHIFT ASSAYS OF THE HYPOXIA-INDUCIBLE FACTOR 1 (HIF-1).

Kvietikova I., Marti H.H., Bauer C., Gassmann M., Wenger R.H.
Physiol. Inst., Universität Zürich, Winterthurerstr.190, CH-8057 Zürich.

Synthesis of erythropoietin (EPO), the primary humoral regulator of erythropoiesis, is upregulated by hypoxia. Recently, a nuclear hypoxia-inducible factor (HIF-1), which recognizes an 8 bp motif within the 3′ flanking region of the EPO gene, has been characterized. Binding activity of HIF-1 is induced in both, EPO-producing and non-EPO-producing cells, suggesting that HIF-1 plays a general role in hypoxic gene regulation. Nuclear extracts from L929 mouse fibroblasts were analyzed by gel shift assays using an oligonucleotide probe containing the core HIF-1 binding site. This probe detected a nonspecific and a constitutive DNA-binding activity in nuclear extracts from normoxic and hypoxic cells, whereas the HIF-1 band appeared only after 1% O_2 induction. Computer analysis revealed homology to a consensus AP-1 site in the vicinity of the HIF-1 binding site. Heterologous competition experiments showed that the constitutive band was efficiently eliminated using a low molar excess of AP-1 oligonucleotide, whereas the HIF-1 band remained unchanged. However, supershift analysis indicate that the constitutive band is not produced by members of the jun or fos families of transcription factors. Further experiments are underway to elucidate the role of the constitutive factor in hypoxic gene regulation.

S01-08

OXYGEN SENSING IN CHEMORECEPTOR CELLS OF THE CAROTID BODY. C. Gonzalez, L. Almaraz, A. Obeso and R. Rigual. Depto. Fisiologia. Facultad de Medicina. Universidad de Valladolid. 47005 Valladolid. Spain.

Chemoreceptor cells of the mammalian carotid body are excitable cells that release dopamine when challanged by low PO_2. The release of dopamine elicited by low PO_2 is Ca^{2+} dependent, modulated by dihydropyridines and inhibited by tetrodotoxin implying that low PO_2 depolarizes chemoreceptor cells. Whole cell recordings made in isolated chemoreceptor cells showed that they possess a transient K^+ current that is reversibly inhibited when PO_2 decreases; equivalent observations were made on recording from isolated patches of membrane. Carbon monoxide prevents the inhibition of the transient K^+ current produced by low PO_2. It is proposed that chemoreceptor cells possess an O_2-sensor in the plasma membrane coupled to the K^+ channel and producing the inhibition of the K^+ current. Additional data suggest that the O_2 sensor is also coupled to an adenylate cyclase via G proteins.
Supported by spanish DGICYT grant PB92/0267.

S01-09

MICROVASCULAR PERFUSION IN SKELETAL MUSCLE AND HEART

Martin F. König, Ewald R. Weibel, and Sanjay Batra
Institute of Anatomy, University of Berne, Bühlstrasse 26, CH-3012 Berne.

An important determinant of gas and metabolite exchange in organs is the fraction of capillaries that are perfused under physiological conditions. Blood plasma was labeled by infusion of 8 nm albumin coated colloidal gold particles into the right atrium of anaesthetized and ventilated rabbits. After 2 minutes of infusion, the circulation was arrested by rapidly closing a snare around the base of the heart; tissue samples were fixed by immersion and further processed for electron and light microscopy. Electron micrographs, and light micrographs of silver enhanced paraffin sections showed colloidal gold particles dispersed in blood plasma. Morphometric analysis at electron microscopic level revealed that 98.3% of the capillary bed of skeletal muscle and 100% of heart capillaries contained gold particles. In both organs, the amount of gold particles per unit plasma varied between capillaries. Whereas in skeletal muscle most capillaries contained only few particles leading to a left shifted curve of the particle distribution, heart tended to reveal a more symmetric plasma particle concentration. From this study we conclude that no relevant part of the capillary bed of soleus and heart remains unperfused within a time span of two minutes. However, differences in the gold particle distribution suggest a different perfusion pattern between muscle and heart presumably based on different critical time intervals for complete filling of the capillary bed, and/or on regional heterogeneity of capillary perfusion velocity. Supported by SNSF grant 31-30946.91

S01-10

EFFECTS OF ACIDOSIS ON THE CONTRACTILITY OF THE EMBRYONIC CHICK HEART DURING NORMOXIA, ANOXIA AND REOXYGENATION

Meiltz A., de Ribaupierre Y., Kucera P. and Raddatz E.
Institut de Physiologie de l'Université, CH-1005 Lausanne.

We investigated whether changes in pH may affect the myocardial function during cardiogenesis to the same extent as in the adult myocardium. The chrono-, ino- and dromotropic effects of external acidosis (pH 6.5) on the activity of the hearts isolated from 4-day-old chick embryos were determined. Under normoxia, acidosis had negative chrono- and dromotropic effects (-20%), decreased transiently the myocardial shortening and the velocity of contraction (-40%) and induced chaotic and periodic bursts of contractions. Hearts were also submitted to successive one-minute episodes of anoxia each separated by 10 minutes of reoxygenation. Anoxia transiently accelerated heart rate by 10 % and 50 % at pH 7.4 and 6.5, respectively and the incidence of the reoxygenation-induced arrhythmias was markedly increased at pH 6.5. Surprisingly, the inhibitor of the Na^+/H^+ exchanger (HMA, 1μM) was not protective at reoxygenation. These findings indicate that the response of the embryonic heart to acidosis resembles that of the adult heart although the underlying mechanisms of pH regulation might be different. S N S F, Grant 31-37668.93.

S01-11

THE REACTIVITY OF THE MYOCARDIUM TO PO_2 CHANGES

Eric Raddatz, *Institut de Physiologie de l'Université, CH-1005, Lausanne.*

The mechanical, electrical and metabolic responses of the myocardium to ischemia and reperfusion constitute a crucial problem in cardiology and have been extensively investigated in a number of experimental models. The major cellular processes underlying the chrono-, ino- and dromotropic effects of graded hypoxia, anoxia and reoxygenation on cardiac activity include energy producing pathways, calcium homeostasis, pH regulation, oxygen radicals production, availability of substrates and gene expression. In order to better understand such mechanisms we have developed an in vitro model of chick embryonic heart characterized by a spontaneous and regular contractile activity and the absence of diffusional limitations allowing a strict control of the PO_2. We found that the embryonic myocardium, which share similarities with the adult conduction system, reacted rapidly and reversibly to changes in PO_2 in between 0 and 100 mmHg and displayed the oxygen paradox phenomenon. Moreover, calcium antagonists, acidosis, antioxidants and glucose concentration modified the pattern of response to anoxia-reoxygenation transitions.
Supported by the Swiss National Science Foundation, Grant 31-37668.93.

S01-12

HYPOXIA INCREASES TRANSCRIPTION OF ERYTHROPOIETIN GENE IN BRAIN AND LIVER OF MURINE FETUSES

R. Kreuter, H.H. Marti, M. Gassmann and C. Bauer
Institute of Physiology, University of Zürich, CH-8057 Zürich

The main physiological regulator of erythropoiesis is erythropoietin (EPO) which is primarily produced in the adult kidney and the fetal liver of mammals. Exposure to hypoxic conditions leads to enhanced EPO production in these organs. Recently, we and others have detected EPO mRNA in the brain of normoxic adult rodents. Furthermore, EPO transcription was increased under hypoxic conditions. Until now, little is known about EPO gene expression during murine development in the brain. Therefore, pregnant C57BL/6 mice were exposed to 0.1% carbon monoxide (a procedure that causes functional anemia) for 6 h or kept at ambiant air. Total RNA was isolated from fetal liver and brain at gestation day 14.5, 16.5 and 18.5. Indeed, at all time points tested EPO mRNA could be detected in both organs of normoxic fetuses by means of reverse transcriptase-mediated polymerase chain reaction (RT-PCR). Competitive RT-PCR analysis revealed an up to 5 fold (day 18.5) increased EPO mRNA level in both liver and brain of hypoxic fetuses compared to the controls, indicating that both organs are able to respond to a reduced oxygen supply. Further quantification of EPO mRNA isolated from earlier fetuses is in progress.

S01-13

Lung diffusing capacity as a function of hematocrit

J. Geiser, P. Choquard, A. Tempini

Institut de Physiologie, Université, 1700 Fribourg

There is no general answer to the question of the optimal value of hematocrit. The present study was undertaken with the aim of assessing the influence of hematocrit on the O_2 transfer between lung alveoli and capillaries. Isolated rabbit lungs (n=12) were connected to an extracorporeal circuit, pump ventilated and perfused with washed bovine erythrocytes at two different hematocrits. Ventilation and perfusion were kept constant when changing hematocrit. All perfusates were adjusted to the same viscosity of 6.5 $m^{-1} \cdot kg \cdot s^{-1}$. The pressures in the pulmonary artery remained unchanged. We can thus expect that ventilation/perfusion mismatch did not change either. When hematocrit was increased from 30 to 70%, an almost proportional increase in O_2 lung diffusing capacity from 0.37 to 0.97 $ml \cdot min^{-1} \cdot mmHg^{-1}$ was observed. We conclude that a) the increase is real and not due to changes in lung inhomogeneity, b) resistance to O_2 transfer in the lung is mainly located in the blood, c) considering only O_2 transfer in the lung, the highest hematocrit values are optimal.

S01-14

Oxygen regulated gene expression: lessons from erythropoietin. P.J. Ratcliffe, Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, OX3 9DU.

Recent work on the regulation of the erythropoietin gene has provided clues to the existence and organisation of a widespread system of gene regulation by oxygen in mammalian cells. Erythropoietin *cis*-acting control (the 3' enchancer) was found to operate in an oxygen inducible manner when transfected into both erythropoietin producing hepatoma cell lines and non-erythropoietin cell lines; physiological characterisation of the response in a wide range of cell types showed features strikingly similar to those established for the native erythropoietin gene (Maxwell *et al*, 1993, PNAS; 90:2423-2427). To follow the implication that the sensing and signal transduction system is operating on other genes in non-erythropoietin producing cells we have examined the regulation of number of genes for similarities with erythropoietin response. We found that the characteristic features of induction by hypoxia, cobaltous ions and iron chelating agents could also be demonstrated for genes encoding glycolytic enzymes and for genes encoding vascular growth factors.

Positive and negative changes in gene expression were observed; in every case responses to hypoxia, cobaltous ions and iron chelating agents were similar in both sign and magnitude. For some genes (phosphoglycerate kinase and lactate dehydrogenese A) we have defined *cis*-acting control elements similar to the hypoxically inducible nuclear factor (HIF-1) binding site in the erythropoietin enhancer. These similarities in both the pharmacological characteristics of gene induction and the regulatory elements provide strong evidence of a common regulatory mechanism, which appears to operate very widely.

Neurobiology of learning

S02-01

RESPONSES OF DOPAMINE NEURONS FOLLOW LEARNING RULES
Mirenowicz J, Schultz, W, Hollerman, JR, Ljungberg T, Romo R (Sponsor J Durand) Inst. de Physiologie, Univ. de Fribourg, 1700 Fribourg.

Previous work indicates that dopamine systems play major roles in the motivational control of voluntary behavior. Learning theories see primary liquid or food rewards as key determinants for controlling basic approach behavior. Neutral stimuli gain the capacity to elicit approach behavior after being paired with primary rewards. The rate of learning is directly related to the unpredictability of reward and reaches an asymptote when reward is entirely predicted by a conditioned stimulus.

We recorded the activity of single midbrain dopamine neurons in monkeys learning and performing controlled behavioral tasks. The majority of dopamine neurons respond selectively to a limited range of stimuli. They are activated by primary rewards as long as these are not predicted in time by a conditioned stimulus. In the presence of a reward predicting stimulus, dopamine neurons respond to it but lose the reward response. The responsiveness to unpredicted rewards and the response transfer to reward predicting stimuli follow basic assumptions of associative learning theories and of efficient neuronal models of reinforcement learning. The dopamine response is sent as a simultaneous population signal via widespread projections to striatum and cortex, appropriate for influencing neuronal processing according to the outcome of behavioral action.

S02-02

NEURONAL ACTIVITY IN PRIMATE STRIATUM DURING LEARNING
Hollerman J, Tremblay T, Schultz W. Institut de Physiologie, Université de Fribourg, 1700 Fribourg.
Striatal neurons were investigated in a learning set paradigm involving a conditional motor task in which two monkeys performed, or refrained from performing, a reaching movement in response to a trigger stimulus depending on a preceding instruction. Three trial types were used, juice-rewarded movement, unrewarded movement and rewarded non-movement. Activity from 196 neurons was recorded using both familiar and new instruction stimuli. Task-related changes included anticipatory activity before and responses after the different task events. Characteristics of activations frequently changed during learning. One type of modification closely followed the subjects changed behavior: neurons with trial selective activity preceding the trigger or reward were frequently activated in inappropriate trials either when the animal chose the incorrect behavioral response or when the animal made a correct movement but appeared to expect a reward in an unrewarded movement trial. The second type of modification involved a loss of selectivity in that normally selective neurons were active in inappropriate trials even when the behavioral response and reward/non-reward associations were correctly made. Activity returned toward that with familiar stimuli after tens of trials. This indicates that the striatum is involved in modified information processing during learning.

S02-03

ANXIOLYTIC-LIKE PROPERTIES OF A SELECTIVE $5HT_{2C}$ RECEPTOR ANTAGONIST IN A VARIETY OF BEHAVIORAL TESTS IN RODENTS.

J.-L. Moreau, M. Bös, , F. Jenck, J.R. Martin, A. Sleight, H. Stadler & J. Wichmann, Pharma Division, Preclinical CNS Research, Bldg. 72/102, F. Hoffmann-La Roche Ltd, Basel 4002.

$5HT_{2C}$ receptors are reported to be involved in the pathophysiology of anxiety disorders. Nonselective $5HT_{2C}$ receptor antagonists such as mianserin and ritanserin have been found to exhibit clinical anxiolytic efficacy. SB 200646A, a selective $5HT_{2C}$ vs $5HT_{2A}$ receptor antagonist (Forbes et al., J. Med. Chem. 36: 1104-1107, 1993), was tested for its binding affinity at the human $5HT_{2C}$ and $5HT_{2A}$ receptors as well as for its potential anxiolytic properties in a number of behavioral paradigms. Membranes of cell lines expressing cloned human $5HT_{2C}$ or $5HT_{2A}$ receptors were used for binding assays. The compound exhibited 16-fold selectivity for the human $5HT_{2C}$ over the human $5HT_{2A}$ receptor. In the light-dark test in mice, the compound (3-30 mg/kg po) increased the number of transitions from one compartment to the other and the time spent in the brightly lit environment without affecting locomotor activity. In the elevated-plus maze in rats (1-10 mg/kg po), it also increased the number of transitions and the time spent in open arms. In an operant conflict test in rats (30 mg/kg po), it increased the number of punished responses received. Finally, in the rotarod and horizontal wire tests in rats, SB 200646A (3-100 mg/kg po) was devoid of motor impairing effects. Taken together, these results suggest that selective $5HT_{2C}$ receptor antagonists may exhibit anxiolytic properties without concomitant side-effects.

S02-04

POTENTIATION AND DEPRESSION OF UNITARY SYNAPSES IN THE RAT HIPPOCAMPUS IN VITRO.

Dominique Debanne, Beat H. Gähwiler and Scott M. Thompson.
Brain Research Institute, University of Zurich, 8029 ZURICH.

Long-term potentiation (LTP) and long-term depression (LTD) in the hippocampus may be induced alternatively at synaptic inputs involving many fibers recruited by the extracellular stimulating electrode. No evidence has been provided, however, that unitary synapses may undergo both potentiation and depression. Pairs of pyramidal neurons in areas CA3 and CA1 of rat hippocampal slice cultures were recorded with potassium methylsulfate-filled sharp microelectrodes. Presynaptic tetani (50 to 100 Hz) induced with 2-5 bursts of action potentials in the presynaptic cell were ineffective in inducing LTP (n = 3). LTP could, however, be induced when single presynaptic action potentials were repeatedly (20-80 x) paired with postsynaptic depolarization (n=10). In 5 pairs, this potentiation was reversed by induction of LTD by repeated (50-100 x) asynchronous pairings of single presynaptic action potentials with postsynaptic depolarization. During LTP, a significant decrease of the synaptic failure rate and an increase in the coefficient CV^{-2} ($mean^2/variance^2$) were observed, suggesting an increase in glutamate release probability. During LTD, in contrast, a significant increase in the failure probability and a decrease in CV^{-2} were observed, suggesting a presynaptic decrease of glutamate release probabililty. Theses results demonstrate that the same synapses may undergo plastic changes in opposite directions and that maintenance of LTP and LTD are partly due to long-lasting modifications of presynaptic glutamate release.

S02-05

DELAYED EMERGENCE OF EFFECTS OF MEMORY-ENHANCING DRUGS: IMPLICATIONS FOR THE DYNAMICS OF LONG-TERM MEMORY.

C. Mondadori, Marion Merrell Dow Research Institute, Strasbourg, France

In laboratory animals improvements of memory can be induced by substances with entirely different primary modes of action. Usually, retention is tested after a lapse of 24 hours or longer, meaning that the drug effects were presumably assessed on the basis of differences in long-term memory. Assuming that differences between treated and untreated animals only become manifest from that moment on when memory is based on the products of the processes modulated by the compounds, comparative studies of the time of appearance of the effects of different substances could furnish information about the dynamics of memory. The present results indicate that the memory-enhancing effects of oxiracetam, arecoline, captopril, and the $GABA_B$ receptor blocker CGP 36742 do not become apparent until 16-24 hours after the learning trial, and are detectable even after retention intervals of up to four months. This indicates on the one hand that the compounds modulate long term memory, and on the other hand, that long term memory comes into play after some 16 to 24 hours. The fact that distinct retention can also be observed after shorter intervals indicates that at these times memory is governed by mechanisms that were not affected by any of the substances tested.

S02-06

THE IMPORTANCE OF OLFACTORY AND VISUOSPATIAL CUES IN A SPATIAL REPRESENTATION.

P. Lavenex, F. Schenk. Institute of Physiology, University of Lausanne.

In this series of experiments, we tried to understand the relationships between visuospatial and olfactory cues in spatial orientation. In particular, we studied the conditions in which both types of cues are used by rats in an eight-arm radial maze.

We found that the use of olfactory cues is critically dependent on the ability of rats to use vision and not on the accessibility of distant visuospatial cues.

When both the visuospatial and the olfactory cues were present and provided contradictory information, rats relied on visuospatial cues. Olfactory cues were not simply neglected, but were considered as secondary information to guide choice.

Under infrared light, rats were able to use olfactory cues in a configurationnal manner for accurate choices, similar to how they use visuospatial cues. However, it appeared that the configuration of the olfactory cues had to be based on a spatial reference and could not be used independently of it.

Rats also proved to be able to use olfactory cues as a list of items for accurate choices, in contrast to visuospatial cues, which are used only in a configurationnal manner.

S02-07

LOCAL KINETICS INFLUENCE GLOBAL SPATIOTEMPORAL ACTIVITY IN A SIMULATED "CORTICAL" MODEL.

S.L. Hill and A.E.P. Villa; Institute of Physiology, University of Lausanne

We have developed a software system for experimenting with the spatiotemporal pattern generation properties in large-scale models of 10^4 cortical neuromimes. The layer consists of two subpopulations of 80% excitatory and 20% inhibitory cells interspersed according to a space-filling pseudo-random distribution. Interconnections are determined randomly according to a Gaussian probability distribution, with the strength of the connections from the inhibitory population set to twice the strength of the excitatory connections. Inhibitory cells are connected over a diameter of 40 cells with a probability of connection which peaks at 20%. Excitatory cells have connections across 20 cells with a maximum probability of connection at 60%. A continuous FitzHugh-Nagumo model, and a discrete leaky integrate-and-fire model are compared based on local phase plots, global activity levels, first-order statistics and the power spectrums of population activities. Our results indicate small differences in the level of activity, firing rate and spatial patterns produced by each model. However, the temporal properties of the spatial patterns are substantially different.

Sponsored by Swiss grant FNRS 31.37723.93.

S02-08

THE NITRIC-OXIDE SYNTHETASE INHIBITOR NITRO-L-ARGININE METHYL ESTER FAILS TO BLOCK CONDITIONED TASTE AVERSION

P. WAELTI, H. WELZL, Behavioral Biology Laboratory, Swiss Federal Institute of Technology, CH-8603 Zürich.

Long-term-potentation (LTP) is a persistent and use dependent increase in synaptic strength that was proposed as model for certain kinds of memory formation [*Nature* **361**, 31(1993)]. Most forms of LTP are known to be NMDA receptor-dependent and there is evidence that non-competitive blockade of this receptor impairs conditioned taste aversion, the learning task used here [*Psychobiology* **18**: 43 (1990)]. Nitric-oxide (NO) is supposed to play an important role as a retrograde messenger in the maintenance of LTP [*Trends Neurosci.* **14**, 60 (1988)]. It was already demonstrated that inhibition of the nitric-oxide synthetase (NOS) prevents some forms of learning [*NeuroReport* 3 (1992), *Proc. Natl. Acad. Sci. USA* **90**, 9191 (1993), *Neuroscience* 52, 393 (1993)] such as spatial-learning in the water maze and olfactory memory in social recognition in rats, conditioned eyeblink in rabbits and taste avoidance in chicks. On the other hand, it does not block shock-avoidance learning in rats.

In order to investigate the role of NO in the acquisition of a novel taste aversion, we injected rats with the NOS-inhibitor w-nitro-L-arginine methyl ester (L-NAME: 50 and 75 mg/kg, intraperitoneally) or saline twice daily for the 4 days before the test. A novel taste (CS: saccharine, 0.1%) was presented to the animals before they were injected with LiCl (UCS, 0.14M, 2% BW) on different delays after the drinking session in order to induce malaise. Forty-eight hours later, the saccharine consumption was measured in a two-bottle choice test (saccharine, vt. tap water). Even with the higher dose, L-NAME treated rats, similar to saline-injected animals showed a delay-dependant avoidance, i. e., with increasing CS-UCS intervals (10 min, 2h, 6h) they decreased their avoidance of saccharine (saccharine consumption expressed as percentage of total fluid intake: 5%, 16%, 75%). We may therefore conclude that NO plays no role in the formation of a new gustatory trace in a taste-aversion paradigm.

S02-09

REWARD PROCESSING IN HONEYBEE LEARNING

M.Hammer, Institut für Neurobiologie, FU-Berlin

In honeybees, an octopaminergic neuron, the VUMmx1 neuron, responds to a sucrose reward, the unconditioned stimulus (US) in olfactory conditioning of the proboscis extension response (PER), with a long-lasting excitation. Since its activity is sufficient to induce PER-conditioning, it can be regarded as a neuronal element mediating the US. The VUMmx1 neuron arborizes in several brain areas involved in olfaction indicating that multiple sites of neural plasticity may underlie the formation of an association. VUMmx1 also responds with a prolonged excitation to an odor (CS) that has been learned to predict the sucrose reward. This response transfer to a CS may provide a neural explanation for some higher-order features of conditioning. Moreover, the response of VUMmx1 to the sucrose reward may depend on a predictive relation between the expected and actual value of the US. VUMmx1's sucrose response is diminished when sucrose is preceded by an odor previously associated with it, but not when preceded by an odor that was not learned to predict sucrose. Thus, the properties of VUMmx1 are compatible with several assumptions of formal theories of associative learning.

S02-10

THEORIES OF LEARNING

A.D. Dickinson, Dept. of Experimental Psychology, University Cambridge, Downing Street, Cambridge CB2 3EB, UK

In this talk I shall review some of the basic connectionist learning algorithms that are claimed to mediate the associative learning observed in conditioning. The fact that associative learning is sensitive to the relative predictive validity of signals of reinforcement favours an error-correcting learning algorithm over a simple Hebbian process. Two ways of implementing such error-correction will be considered: McLaren's circuit for deriving an error signal and Konorski's suggestion that connection plasticity is sensitive to derivatives of unit activity. Studies of the effect of'surprising' post-trial events demonstrate that associability processes, but at present it is not clear how to implement such processes within a connectionist system.

Modelling and therapy of genetic disorders

S03-01

GDNF: A NEW NEUROTROPHIC FACTOR FOR MOTONEURONS EXPRESSED IN MANY SUBPOPULATIONS OF NEURONS IN THE BRAIN.

N. Hoffmann-Pochon, S.A. Tan, P. Aebischer, A.D. Zurn Division de Recherche Chirurgicale, CHUV, 1011 Lausanne.

Glial cell line-derived neurotrophic factor (GDNF) has recently been postulated to be a dopaminergic neuron-specific neurotrophic factor (Lin et al., 1993). We report here that GDNF released by GDNF-transfected Baby Hamster Kidney (BHK) cells induces a 5-6-fold increase in the activity of choline acetyltransferase (ChAT) in cultures from the embryonic rat ventral mesencephalon and spinal cord, but not in septal, striatal, and pedunculopontine cholinergic neurons *in vitro*. Furthermore, we show that the local application of polymer-encapsulated BHK cells releasing GDNF to transected facial nerve in newborn rats diminishes the death of motoneurons normally occurring after axotomy in the neonatal period. The present results suggest that GDNF may be a potential therapeutic agent in the treatment of motor neuron diseases such as amyotrophic lateral sclerosis.
To further determine the role of GDNF on different neuronal subpopulations, we are analyzing the expression of GDNF using PCR and in situ hybridization. Embryonic rat striatum, substantia nigra, cortex, spinal cord, cerebellum, thalamus, but also muscle, cartilage, lung and kidney were analysed. Using both in situ hybridization and immunocytochemistry, we have identified subpopulations of neurons labelled for GDNF and glutamate decarboxylase (GAD) in the adult rat striatum and GDNF and tyrosine hydroxylase (TH) in the substantia nigra. We are currently analyzing the possible regulation of GDNF transcription in the brain stem and spinal cord after axotomy of the facial and sciatic nerves.

S03-02

OPTIMIZING THE CONDITIONS FOR TRANSFECTION OF MELANOMA CELLS BY ELECTROPORATION

Döbbeling, U., Davis-Daneshfar, A., Dummer, R., and Burg, G, Universitätsspital Zürich, Dermatologische Klinik, Gloriastrasse 31, CH-8091 Zürich, Switzerland.

"Infection" with retroviral vehicles has been widely used to transfect cancer cells with genes to make these cells more immunogenic (e.g. cytokines) for the purpose of developing cancer vaccines. Although this method has been proven to be save and successful, some patients fearing potential biohazards remain sceptic to use this method. For this reason we have investigated the potential of electroporation to transfect cancer cells. In a first step we have determined the optimal electroporation conditions for the melanoma cell line SK-MEL. Using 10^7 cells and 20 mg/ml PBS, we found that an electric field strength of 1.1-1.3 kV/cm was optimal for both transient and stable transfection of this cell line. We are currently investigating the ex-pression of the transfected genes (IL-2, B7) and the optimal electroporation conditions for cell lines freshly derived from melanoma metastases.

S03-03

DISTINCT DELETIONS AND MUTATIONAL HOT SPOTS WITHIN THE CARBOXY TERMINAL REGION OF THE LMP1 ONCOGENE OF EPSTEIN-BARR VIRUS ARE FREQUENTLY IDENTIFIED IN LYMPHO-PROLIFERATIVE DISORDERS.

S.Rothenberger, E.Bachmann, H.Knecht.* DMI, CHUV. Lausanne, *University of Massachusetts Medical Center, Worcester, MA.

Latent membrane protein-1 (LMP1) is an oncogene expressed by Epstein-Barr virus (EBV) in Reed-Sternberg cells, nasopharyngeal carcinoma, and several human lymphoma cells. We have recently identified in aggressive Hodgkin's disease (HD) a LMP1 variant characterized by 4 point mutations and a 30 bp deletion. To test whether such mutants were also present in other lymphoproliferative disorders (LPD) we screened 104 EBV positive human DNA samples by PCR. DNA sequencing of the region coding for the carboxy terminal domain was performed on 30 samples. In 19 samples a combination of 4 point mutations was identified; among these, 14 had a 30 bp deletion. One sample presented a 69 bp deletion and 4 point mutations. Deletion mutants were found in HD, angioimmunoblastic-lymphadenopathy, B-immunoblastic lymphoma, centroblastic lymphoma, peripheral T cell lymphoma, chronic juvenile LPD and two lymphoblastoid cell lines from cancer patients. Our findings reveal a high frequency of mutations at preferential sites within the carboxy terminal domain of the LMP1 oncogene, in a region critical for the half-life of the protein. The association of these mutational hot spots with LPD suggests that they are involved in EBV related lymphomagenesis and they define a clinically relevant EBV strain.

S03-04

Adenovirus protease L3/p23 involved in virus entry and uncoating

U. Greber, P. Webster, J. Weber[1] and A. Helenius
Yale University School of Medicine, New Haven CT 06520 USA and [1] University of Sherbrooke, Canada

The identification of genes responsible for aquired and inherited diseases has generated great expectations for the molecular treatment of somatic gene deficiencies. The major bottleneck towards satisfying these expectations is, however, the lack of effective and safe gene delivery systems for correct targeting and efficient gene delivery to the cell nucleus. Viruses have evolved highly efficient mechanisms for entry and gene delivery into cells and many recombinant viral vectors, in particular adenoviruses, are currently used for gene delivery into somatic cells. My goal is to understand the entry and uncoating mechanisms of adenovirus. Our previous results indicated that during cell entry the adenovirus disassembles in a stepwise process, degrades or sheds selective capsid proteins and finally releases the genomic DNA into the nucleus. Using quantitative biochemical, immunochemical and morphological methods we now show that inhibitors of the viral cysteine protease L3/p23, located inside the capsid, prevent the degradation of the incoming protein VI and hexon and diminish cell infection. Inhibitor-treated virus internalizes into cells and localizes to nuclear pore complexes like control virus, but is unable to uncoat and translocate capsid proteins into the nucleus. The protease-defective ts1 mutant virus does not degrade the precursor of protein VI and does not disassemble. Protein VI degradation inside the capsid of wild type virus, but not of protease-inactivated virus, can be triggered by a plasma membrane fraction requiring the cellular internalization receptor, a fibronectin-binding integrin, and the viral receptor ligand, penton base. The data indicates that virus-receptor interactions reactivate the adenovirus protease enabling efficient virus uncoating and cell infection.

S03-05

TOWARDS A GENETIC THERAPY OF CHRONIC GRANULOMATOUS DISEASE

J.P. Hossle*, M. Grez‡, S. Schneider*, C. Barbey$, S. Rusconi#, W. Schaffner+, and R.A. Seger*. *University Children's Hospital, Zurich; ‡Georg-Speyer-Haus, Frankfurt; $Institute for Biochemistry, University of Lausanne; #Institute for Biochemistry, University of Fribourg; +Institute for Mol. Biol. II, University of Zurich

Chronic granulomatous diseases (CGD) are immunodeficiencies characterized by a predisposition to life-threatening recurrent bacterial and fungal infections. The underlying cause are defects in phagocyte NADPH oxidase subunits, leading to absence or malfunction of microbicidal killing. Close to 70% of CGD patients suffer from the X-linked form of the disease, characterized by defects of the gp91-*phox* subunit. The gp91-*phox* gene has been identified, sequenced, cloned, and characterized. This project is directed at the development of clinically applicable protocols for transitory and permanent somatic gene therapy of CGD. In a complementary approach recombinant adenovirus vectors as well as retroviral vectors are constructed. Adenovirus-mediated gene transfer into terminally differentiated phagocytes is envisaged as transitory therapy in order to overcome severe acute infections, whereas retroviral vectors should allow for permanent somatic gene transfer into hematopoietic precursor cells. Preliminary results demonstrate successful adenoviral gene transfer into human macrophage-like cells. At least partial reconstitution of NADPH oxidase activity in EBV-transformed CGD B- cells was observed after retroviral infection with recombinant gp91-*phox* murine stem cell virus (MSCV).

S03-06

NEUROTOXICITY INDUCED BY ß-AMYLOID LEADS TO EITHER NECROTIC OR APOPTOTIC CELL DEATH DEPENDING ON CELL TYPE.

Gschwind M. and Huber G., *Pharma Division, Preclinical Research, F. Hoffmann-La Roche Ltd, CH-4002 Basel.*

A major component of senile plaques in the brain of Alzheimer's disease (AD) patients is the amyloid ß peptide (Aß), a proteolytic fragment of the ß-amyloid precursor protein (ß-APP). Surrounding these senile plaques increased neuronal cell loss is found and there is evidence that Aß is directly neurotoxic. In the present study four different neuroblastoma cell types were treated for one day with human $Aß_{1-42}$, which is mainly present in the brain of AD patients. Rat PC12 and human IMR32 cells were most susceptible to Aß-induced toxicity although the peptide was found to strongly reduce viability in all cell types tested. Chromosomal condensation and fragmentation of nuclei, features indicating apoptotic cell death, were seen in PC12, NB_2a and B104 cells but not in IMR32 cells irrespective of their high sensitivity to Aß. In addition, electrophoretic analysis of cellular DNA confirmed internucleosomal DNA fragmentation typical for apoptosis in all cell types except IMR32. These findings suggest that Aß may induce either necrosis or apoptosis depending on the cell type.

S03-07

NEURONAL GROWTH INHIBITORY FACTOR, GIF (OR METALLOTHIONEIN-3), BINDS ZINC MORE STRONGLY THAN Aβ PEPTIDE

Pountney, D.L., Faller, P., Bogumil, R. & Vašák, M. Biochemistry Institute, University of Zürich, CH-8057 Zürich.

Alzheimer's disease (AD) brain is characterized by extracellular plaques of insoluble amyloid, composed mainly of Aβ peptide. Aggregation of Aβ is promoted by Zn binding specifically to the peptide (K_A, 10^7 M^{-1}), linking interneuronal plaque formation to the unusually high concentrations of Zn found in the AD brain. However, little is known about the speciation of neural Zn. Neuronal growth inhibitory factor, GIF, (7 kDa) contains 1-4 tightly-bound Cu(I) and 3-5 labile Zn(II) ions, is colocalized with the pre-synaptic Zn vesicles and is depleted in the AD brain. We have determined by potentiometric titration and competition experiments that GIF binds Zn (K_A, 10^{12} M^{-1}) at least 10^5 times more strongly than Aβ. Therefore, Zn sequestered in GIF (normally *ca.* 3 µg protein/g tissue) will be unavailable to Aβ and the reduced GIF concentrations in AD brain may lead to increased free Zn levels. Further studies on Zn speciation in the brain are in progress.

S03-08

GENE THERAPY FOR PRIMARY IMMUNODEFICIENCY DISORDERS

C Kinnon, Institute of Child Health, London, UK.
We are attempting to optimise the introduction of genes into bone marrow progenitor and stem cell populations, with the aim of developing techniques which will be applicable generally for the treatment of all primary immunodeficiency and other bone marrow related disorders by somatic gene therapy. We have concentrated so far on developing a model system, using retroviral vectors to treat chronic granulomatous disease (CGD). Our results suggest that although we are able to transduce progenitor cell populations with relatively high efficiency, transduction of true stem cell populations is a more elusive goal. These results have been borne out by our clinical trial of somatic gene therapy, for adenosine deaminase (ADA) deficient severe combined immunodeficiency (SCID). Since adeno-associated viruses (AAV) have the advantage of being able to transduce quiescent stem cell populations with high efficiency, we are now developing AAV vectors for use in such treatment.

S03-09

DEVELOPMENT OF HIGH SAFETY ADENOVIRAL VECTORS

Stefano Brenz Verca and Sandro Rusconi, Institute of Biochemistry, Rue.du Musée 5, 1700 Fribourg.

The conventional approach for the generation of recombinant adenovirus consists in cotransfecting the DNA of a replication-defective adenovirus missing at least the E1 region and a recombination plasmid bearing the gene to be expressed into a helper cell line complementing the missing regions. Viral growth is thereby theoretically restricted only to such a helper cell line.However there are several widespread human and animal viruses that encode factors potentially able to complement these defects.We are currently introducing some modifications in this recombination system in order to eliminate the residual viral replication potential and to facilitate the detection of recombinants. For this purpose we are knocking out the virus-encoded DNA polymerase (Ad pol) and making the MLP-directed (Major Late Promoter) transcription of late genes dependent from a transcription factor not occuring in higher eukaryotes. An E1-transformed cell line stably expressing Ad pol and the non-ubiquitous transcription factor will be essential for the replication of this adenovirus. The second major modification involves the use of LacZ α-complementation analogous to that known in prokaryotes (see abstract by Moosmann et al). Our recombination vectors will encode for a dicistronic expression unit consisting of the gene to be expressed followed by the LacZ α-peptide translated under the control of a picornaviral internal ribosomal entry site (IRES).

S03-10

Targeted disruption of the *pmp22* gene in mice as a model of nerve degeneration in human.

Katrin Adlkofer, Rudolf Martini, Adriano Aguzzi[#] & Ueli Suter
Swiss Federal Institute of Technology, ETH Hönggerberg & [#]University of Zürich, Switzerland
The peripheral myelin protein 22 (PMP22) is a hydrophobic glycoprotein of 160 amino acids which is highly expressed in the cell membrane of mature Schwann cells and appears to play an important role in the molecular pathogenesis of hereditary peripheral neuropathies in mice and humans. In particular, point mutations in one of the four putative transmembrane domains lead to autosomal dominant diseases in *trembler* and *trembler-J* mice. In the hereditary human hypertrophic neuropathy Charcot- Marie Tooth disease type 1A (CMT1A), 1.5 megabases of chromosome 17, including the *PMP22* gene, are duplicated. Deletion of the same region is associated with the human hereditary neuropathy with liability to pressure palsies (HNPP). In both diseases the myelination of the peripheral nerves system is highly affected. Suggested functions of the PMP22 protein include cell adhesion and cell-cell communication processes. Alternatively, PMP22 may have also functions in regulation of cell growth .
In an attempt to elucidate the function of PMP22 in normal and diseased tissue, we have generated mice homozygous for a disrupted *pmp22* gene. Preliminary analysis indicates that the heterozygous disruption of *pmp22* provides a mouse model for the human neuropathy HNPP. Furthermore mice which are completely deficient of *pmp22* exhibit a strong phenotype including severe hypermyelination and reduced numbers of of axons in the peripheral nervous system.

S03-11

DEFECTIVE KERATINOCYTE TRANSGLUTAMINASE CAUSES LAMELLAR ICHTHYOSIS

Huber M., Rettler I., Bernasconi K., Frenk E., Lavrijsen S.+, Ponec M.+, Schorderet D.§, Hohl D.; Dermatology and §Unit of Molecular Genetics, CHUV, Lausanne; +Dermatology, Leiden.
Transglutaminases (TG) constitute a homologous gene family of enzymes cross-linking proteins by transamidation of glutamines. Different members of this family catalyze blood clotting, apoptosis, signal transduction due to additional GTPase activity, formation of epidermal cornified cell envelopes and keratinization of hair. Recently, we observed altered epidermal expression of keratinocyte TG1 in autosomal recessive lamellar ichthyosis (LI) suggesting that defective TG activity could cause the disease characterized by scales covering the whole body. Cells cultured from 5 LI patients had reduced TG1 activity (0-5% of normal), abnormal mRNA and faulty protein synthesis. SSCP and sequencing of genomic DNA identified mutations in the TG1 gene which either cause aberrant splicing, a shift in the reading frame or change highly conserved amino acid residues. This demonstrates that LI is caused by missing protein cross-linking activity during epidermal differentiation and that intact TG1 contributes to the homeostasis of the outer surface of the skin. The identification of the gene responsible for LI opens up the possibility of using gene therapy in these patients.

S03-12

INTRACELLULAR LOCATION OF ß-AMYLOID FORMATION

G. Schrader-Fischer and P.A. Paganetti; Sandoz Forschungsinstitut Bern, P.O.Box, 3001 Bern

ß-amyloid (ßA4) mediates the pathogenesis of Alzheimer's Disease (AD). The cellular mechanisms regulating ßA4 generation from the ß-amyloid precursor protein (APP) are not well understood. We investigated the cellular processing of APP to ßA4 in human cell lines stably expressing different APP cDNA constructs. We generated APP constructs lacking sorting domains at the C-terminus as well as APP chimeric proteins carrying heterologous sorting signals. We compared these constructs to familial AD APP mutants (e.g. Swedish APP mutation). None of the APP constructs led to the same high level of ßA4 secretion obtained with the Swedish APP mutation (5-8 fold if compared to wild-type APP). The cDNA construct with a truncated APP molecule led to an increased secretion of a shorter fragment produced by an alternative processing pathway. APP chimerae carrying lysosomal targeting signals showed a reduction of ßA4 formation. We propose that subcellular compartments such as endosomes or lysosomes are not necessary for ßA4 formation and secretion.

S03-13

AN EPISOMAL VECTOR IN MOUSE EMBRYONIC STEM CELLS AND DIFFERENTIATED RODENT CELL LINES

Gruber M.[1], Donoho G.[2], Berg P.[2] and Gassmann M.[1]
[1]Physiologisches Institut, Universität Zürich, Winterthurerstr. 190, 8057 Zürich. [2]Department of Biochemistry, Beckmann Center, Stanford University Medical School, Stanford, CA 94305-5425

We have constructed a polyoma virus-based plasmid vector that is episomally maintained in embryonic stem (ES) cells without altering their capability to populate an embryo. The plasmid vector pMGD20*neo* contains the polyoma origin of replication, a modified gene encoding the large-T protein and a gene that confers resistance to G418 (*neo*). The question arose whether this vector was able to be maintained episomally in different cell lines and whether insertion of a similar sized DNA sequence would impair the vector's replication capability. Therefore, we transfected several cell lines either with pMGD20*neo* (7.2 kb) or with a pMGD20*neo*-derived construct harboring a 6.8 kb fragment encompassing exons 2 and 3 from the *hprt* gene. Efficient replication of both vectors was observed in ES-cells, mouse embryonic carcinoma cells (F9), mouse adenocarcinoma (RAG) cells and, to a lesser extent, in mouse erythroleukemia (MEL) cells, but was not detected in mouse L-fibroblasts (L929), rat aortic endothelium carcinoma (RAEC) cells and primary embryonic fibroblasts. At present, we are testing if the efficiency of mutating chromosomal genes in ES cells can be improved by maintaining the targeting sequence on this episomal vector. To this end, we attempt to disrupt the X-linked *hprt* gene using the pMGD20*neo-hprt* targeting vector described above.

S03-14

TRANSGENIC MODELS OF ALZHEIMER'S DISEASE

B. Ledermann, Preclinical Research, Sandoz Pharma Ltd., CH-4002 Basel

Alzheimer's disease (AD) is a progressive neurodegenerative disorder and the most common form of dementia as it accounts for around 50 % of all cases of dementia in the industrialized world. The two main neuropathological hallmarks found in AD brains are extracellular amyloid plaques and intracellular neurofibrillary tangles (NFT's). Amyloid plaques contain insoluble deposits of β-amyloid, a fragment of the ubiquitous amyloid precursor protein (APP). Neurofibrillary tangles are composed of insoluble paired helical filaments formed by the abnormally phosphorylated protein Tau that is normally associated with microtubules. Several animal models, including app- and tau-transgenic mice, have been generated in order to evaluate the mechanisms of plaque and NFT-formation. Human App751-transgenic mice show extracellular β-amyloid deposits that increase in frequency with age. Tau-transgenic mice show high expression of human tau in a small percentage of nerve cells but no NFT's have been observed so far. In addition, mice that are trisomic for a segment of mouse chromosome 16 that is conserved in human chromosome 21 are being studied to clarify molecular mechanism involved in AD since human individuals with trisomy 21 (Down syndrome) develop AD pathologies at early age.

S03-15

ANALYSIS OF THE EPITHELIAL SODIUM CHANNEL (ENac) IN TRANSGENIC MICE

Hummler,E.[1], Verdumo,C.[1], Beermann,F.[2] and Rossier,B.[1] Institute of Pharmacology and Toxicology[1], Lausanne; Swiss Institute for Experimental Cancer Research[2] (ISREC), Epalinges, Switzerland

Transport of Na^+ ions through the epithelial Na^+ channel (α,β and γENac) is the rate-limiting step of Na^+ absorption and thereby controls osmotic balance of body fluids and secretions. Recent studies demonstrated that mutations in the β subunit of this channel cause hypertension (Liddle's syndrome) probably due to a constitutive activation of the channel (Cell 79, pp 407, 1994).
In order to elucidate the role of this channel in vivo we generated transgenic constructs in which α,β and γrENac are linked to the human CMV promoter.
Several transgenic lines showing expression in different tissues were established. Progress in the analysis of those mice will be discussed.

S03-16

The amino terminal segment of the mature PrP protein is not necessary for scrapie pathogenesis and replication

M. Fischer, A. Raeber, T. Rülicke+, S. Brandner*, A. Aguzzi* and C. Weissmann. *Institut für Molekularbiologie I der Universität Zürich, Hönggerberg, 8093 Zürich, +Biologisches Zentrallabor and * Institut für Neuropathologie, Universitätsspital Zürich, 8091 Zürich*

PrP^C is a protein normally expressed in the brain and other tissues of all vertebrates examined. Scrapie-infected brain contains modified, partially protease-resistant form(s) of PrP^C designated PrP^{Sc}. The infectious agent of scrapie, the prion, is protease-resistant and thought to be PrP^{Sc} or a subset of it. We have shown that in PrP knockout mice pathogenesis and replication of the scrapie agent are abolished. Susceptibility can be restored by introducing PrP transgenes, and incubation times are inversely correlated to PrP expression levels. Here we report that PrP genes encoding PrP with amino terminal deletions of up to 23% percent of the mature protein (which comprises 208 amino acids) still restore susceptibility to scrapie and allow prion replication. The expendable PrP segment corresponds to the PrP^{Sc} domain remaining susceptible to protease.
Unexpectedly, in contrast to $PrnP^{+/+}$ mice, transgenic animals overexpressing normal or mutant PrP showed no increase in protease-resistant PrP upon acquisition of scrapie symptoms.

S03-17

Deletion of a >300-kb amyloid precursor protein (APP) gene segment in mouse ES cells using cre recombinase-mediated recombination

Z.-W.Li, U.Müller, J.Götz, G.Stark, T.Rülicke* and C.Weissmann
*Institut für Molekularbiologie I, Universität Zürich, 8093 Zürich, *Biologisches Zentrallabor, Universitätsspital Zürich, 8091 Zürich, Switzerland*

Homologous recombination allows the generation of mutations, in particular disruptions and deletions, in murine ES (embryonal stem) cells which can then be used to generate homozygously mutated mice. Deletions of up to about 20 kb have been reported, but we were not successful in deleting a segment of 300-400 kb of the APP gene using the classical gene targeting procedure (Capecchi). We therefore developed a cre recombinase-dependent strategy. First, a DNA segment containing the elements neo-lox-tk (neo=neomycin resistance gene, lox=target sequence for the cre recombinase, tk=HSV thymidine kinase gene) was introduced immediately downstream of exon 2. In a second step exon 17 was replaced by tk-lox-hygro (hygro=hygromycin resistance gene). A cre expression plasmid was then introduced into ES cells containing the two lox sequences (in the same orientation) by electroporation. After exposure to FIAU, which kills tk-containing cells, all surviving clones had undergone deletion of the gene segment between the lox sequences, as evidenced by PCR and Southern analysis.

S03-18

Behavioral and anatomical deficits in mice homozygous for a modified β-amyloid precursor protein (APP) gene

U. Müller, N. Christina, Z.-W. Li, D. P. Wolfer*, H.-P. Lipp*, T. Rülicke+, S. Brandner$, A. Aguzzi$ and C. Weissmann. *Inst. für Molekularbiologie I, and *Anatomisches Inst., Universität Zürich, +Biologisches Zentrallabor and $Inst. für Neuropathologie, Universitätsspital Zürich, Switzerland*

The occurrence of familial forms of Alzheimer's disease that are linked to mutations in the β-amyloid precursor protein (APP) gene suggests that APP and its metabolism plays an important role in the pathogenesis of the disease. To determine the physiological role of APP we disrupted the murine APP-gene by inserting into exon 2 a cassette containing a neomycin resistance gene and a putative transcription termination sequence. Contrary to expectation, brain and other tissues from mice homozygous for the insertion still contained APP-specific RNA, albeit at a level 5-10 fold lower than wild-type and lacking the disrupted exon, which had been spliced out. The brain contained shortened APP-specific protein at a low level. Mutant mice were severely impaired in spatial learning and exploratory behavior (showing passivity in a novel situation and strongly reduced locomoter activity) and had increased incidence of agenesis of the corpus callosum. Generation of a large genomic deletion encompassing most of the coding sequence is currently underway. Since APP belongs to a family of homologous proteins (APP-like proteins, APLPs), that may also be functionally related, we are presently generating null mutation in these genes as well.

S03-19

EXTENT OF THE PLEIOTROPIC RESPONSE OF HEPATOCYTES TO PHENOBARBITAL

F.Früh, and U.A.Meyer; Biocenter of the University, Department of Pharmacology, CH-4056 Basel

Phenobarbital (PB) induces the expression of liver enzymes involved in the metabolism of drugs and other foreign compounds (xenobiotics). These enzymes include several cytochromes P450, NADPH:P450 reductase, aldeyde dehydrogenase, epoxide hydrolase, UDP-glucuronyltransferases and glutathione-S-transferases. Many other proteins not yet recognized may however also be induced by PB. The extent of this pleiotropic response and the molecular mechanism of induction remain unknown.
Differential-Display (DDRT-PCR) of non-induced vs induced chicken embryo liver mRNA indicates that PB treatment results in the transcriptional activation and repression of >20 genes in a ratio of 2:1 (induction vs repression). Several of these PCR products were subcloned and further analyzed. Sequence information derived from these PCR products revealed genes known to be induced by PB (i.e. CYP2H1 and GST) as well as a variety of new induced or repressed genes.

S03-20

Distinct Immundeficiencies in TNF/LTa double deficient mice

Matthias Müller, Hans-Pietro Eugster, Bruce D. Car , Urs Karrer ‡, Bruno Schnyder, Vicki M. Eng , Gaetane Woerly, Michel Aguet†, Rolf Zinkernagel ‡ Horst Bluethmann ♦ and Bernhard Ryffel
Swiss Federal Institute of Technology Institute of Toxicology Schorenstrasse 16, CH-8603 Schwerzenbach, ♦ Department of Genetics, Pharmaceutical Research, F. Hoffmann-LaRoche Ltd, CH-4002 Basel, ‡ Institute for Experimental Immunology, Schmelzbergstrasse 12, 8091 Zürich, † Institute of Molecular Biology I, University of Zürich, Hönggerberg, 8093 Zürich, Switzerland (present address: Genentech Inc., 460 Point San Bruno Blvd. South San Francisco, CA 94080, USA)

Tumor necrosis factor (TNF) and lymphotoxin alpha (LTa) are pleiotropic and homologous cytokines involved in host defence and the pathogenesis of different diseases. In order to investigate their roles in the development and function of the immune system, the *Tnf* and *Lta* genes were simultaneously inactivated in mice by homologous recombination. These mutant mice combine the phenotypes of TNFR1-knockout and LTa-knockout mice comprising susceptibility to Listeria monocytogenes infection, resistance to endotoxic shock and absence of lymph nodes and Peyer's patches. In addition, the mutant mice lack intestinal IgA producing plasma cells, show immunoglobulin depositions in the renal glomerulus and an upregulation of MHC class I and II antigen expression in different tissues in the presence of an absolute B lymphocytosis and mixed lymphocyte infiltrates in different tissues. These data suggests important and specific roles for both ligands in the development of a functional immune system.

S03-21

ANTISENSE INHIBITION OF THE MESODERM-DETERMINING GENES *BRACHYURY* AND *XHOX3* IN *XENOPUS* EMBRYOS

Giovannini, N., and Rungger, D., Station de Zoologie expérimentale, Université de Genève, 1224 Chêne-Bougeries

An expression vector read by RNA polymerase III produces high amounts of antisense RNA in developing *Xenopus* embryos between mid-blastula transition and early tailbud stage. Using this tool, we have tried to inhibit the expression of the *brachyury (Xbra)* and *Xhox3* genes that are involved in mesoderm determination and patterning. In part of the embryos carrying the antisense vector directed against Xbra mRNA, the target protein is reduced or its expression locally disturbed. As a consequence, the embryos develop heavily deficient phenotypes lacking either anterior or posterior structures, exhibiting axis distortion, or failing differentiation completely. The embryos subjected to antisense treatment against Xhox3 mRNA, show similar defects in their organisation which agrees with the fact that both genes cooperate in patterning the developing mesoderm.

S03-22

SELECTION OF RECOMBINANT BOVINE HERPESVIRUS 5 gC DELETION AND REPLACEMENT MUTANTS

Monika Engels, Paul Hecht, Eva Loepfe and Mathias Ackermann
Institute of Virology, Vet.med. Faculty, University of Zürich, Winterthurerstr.266a, CH-8057 Zürich

Bovine herpesvirus 5 (BHV5) is closely related to bovine herpesvirus 1 (BHV1), but differs from BHV1 by its neuropathogenic potential. Mechanisms being responsible for the pathogenetic differences are mostly unknown. One viral factor which might influence the outcome of the infection is the envelope glycoprotein gC. It is involved in the adsorption process, but is non-essential for virus replication and therefore supposed to mediate a "luxury" function.

In order to study those functions, we have cloned and sequenced the BHV1 and BHV5 gC genes. In addition we constructed cassettes containing either the β-galactosidase (β-gal) gene or the heterologous gC gene flanked by BHV1 or BHV5 specific sequences for homologous recombination after cotransfection with genomic DNA in MDBK cells.

We were able to select two recombinant viruses (rBHV5), as confirmed by DNA and protein analyses. Blue plaques representing rBHV5 ΔgC-blue, carrying the β-gal instead of the gC gene, were selected following Bluogal staining. Compared to its parent the recombinant virus showed a reduced growth rate and a different plaque morphology. An intertypic recombinant, rBHV5/gC$_1$, expressing BHV1 gC (gC$_1$) in the place of gC$_5$, was selected by a magnetic separation system (MiniMacs, Miltenyi Biotec Inc.) using a gC$_1$ specific monoclonal antibody. Growth characteristics of this recombinant did not obviously differ from its parent. These recombinants will help to analyze the influence of gC in the process of initiating the BHV infection *in vitro* and *in vivo*.

S03-23

THE POTENTIAL ROLE OF RECOMBINANT PHAGE-DISPLAY ANTIBODY LIBRARIES IN THE EXAMINATION OF DRUG-INDUCED AUTOIMMUNITY

Urs Christen and Josef Gut; Department of Pharmacology, Biocenter of the University, CH-4056 Basel, Switzerland

Autoimmunity induced by drugs is a widespread phenomenon; in the case of hepatitis caused by the anesthetic agent halothane the targets for autoantibodies, occurring in patients suffering from the disease, consist of trifluoroacetyl-protein adducts (CF_3CO-proteins). These protein modifications arise upon oxidative, cytochrome P-450-dependent metabolism of halothane in *all* exposed individuals. However, only a small subset of *susceptible* individuals develops a fulminant hepatitis. One possible reason for the obvious *tolerance* of CF_3CO-proteins in the vast majority of individuals is the existence of *molecular mimicry* of CF_3CO-lysine by *lipoic acid* which is constitutively present as the prosthetic group of the E2 subunits of the 2-oxoacid dehydrogenase complexes. The concept of molecular mimicry by endogenous structures as a protection mechanism against autoimmunity to modified self-proteins might be extended to other potential neoantigens arising upon exposure to drugs or environmental agents. A powerful tool for the identification of structures which confer molecular mimicry are monoclonal antibodies which are not able to discriminate between an endogenous structure and a neoantigen. Such cross-reactive monoclonal antibodies can be provided by a recombinant phage-display antibody library which allows the screening and selection of monoclonal antibody-fragments for reactivity towards antigens of interest.

S03-24

TUMOR CELL TARGETTING OF VACCINIA VIRUS EXPRESSING A SINGLE CHAIN Fv ANTIBODY

M. Galmiche*, L. Rindisbacher#, W. Wels°, R. Wittek# and F. Buchegger*, Instituts de *Biochimie et de #Biologie animale, Université de Lausanne and °Klinik für Tumorbiologie, Freiburg, Germany

The tumor antigen erbB-2 is overexpressed in some of the breast and ovarian tumors. Grafting of an anti-erbB-2 scFv onto cytotoxic T-lymphocytes specifically targeted the cytolytic activity to erbB-2 expressing tumor cells. We want to use a similar approach to target vaccinia virus to cancerous tissues. Specifically, expression of an anti-erbB-2 antibody on the surface of a recombinant vaccinia virus might preferentially bring the virus into contact with the tumor cells, which would be infected and destroyed.

Vaccinia hemagglutinin is expressed on the surface of extracellular enveloped virus. Deletion experiments revealed that the protein is not essential for virus replication and infectivity. We are therefore replacing various domains of vaccinia virus strain IHD-J hemagglutinin by an anti-erbB-2 single-chain antibody (scFv). The recombinant viruses expressing the scFv on its surface will be selected by panning on an anti-idiotype antibody. The infectivity of the different recombinants will be tested on cells expressing or not the erbB-2 antigen.

S03-25

EXPRESSION OF ORNITHINE TRANSCARBAMYLASE IN E.COLI

Oppliger Leibundgut E., Liechti-Gallati S.[1] and Wermuth B.; Departments of Clinical Chemistry and [1]Clinical Pharmacology, University of Berne.

Ornithine transcarbamylase (OTC), the 2nd enzyme of the urea cycle, is encoded by nuclear DNA and processed after import into the mitochondrion. OTC deficiency is the most common inborn error of ureagenesis. About 80 point mutations of the OTC gene have been identified; however most of them await proof of being deleterious rather than innocent polymorphisms. To this end we have cloned the cDNA coding for the mature protein into the pET-11a expression vector. Expression of the recombinant protein in E. coli yielded a product that was enzymatically active although a substantial part was insoluble. The supernatant was purified by ion exchange and gel chromatography. Km values for carbamyl phosphate and ornithine were 0.25 and 0.5 mM, respectively, in good agreement with the values of the native enzyme. Expression of a functional OTC in E.coli allows to study the effect of mutations by site-directed mutagenesis.

S03-26

THE *DROSOPHILA* TUMOR SUPPRESSOR GENE, *WARTS (WTS)*, ENCODES A HOMOLOG OF HUMAN MYOTONIC DYSTROPHY PROTEIN KINASE (DM-PK)

Olav Zilian[1], Robin W. Justice[2], Daniel F. Woods[2], Peter J. Bryant[2], and Markus Noll[1]
[1]Institut für Molekularbiologie II, Universität Zürich Irchel, 8057 Zürich
[2]Developmental Biology Center, University of California, Irvine, CA 92717

Homozygous loss of the *warts* gene of *Drosophila*, caused by mitotic recombination in somatic cells, leads to cell clones that are fragmented, rounded, and overgrown compared to normal controls. The gene is therefore required for the control of the amount and direction of cell proliferation as well as for normal morphogenesis. The absence of *wts* function also results in apical hypertrophy of imaginal disc epithelia. Secretion of cuticle over and between the domed apical surfaces of these cells leads to a honeycomb-like structure and gives the superficial wart-like phenotype of mitotic clones in the adult. One *wts* allele allows survival of homozygotes to the late larval stage and these larvae show extensive imaginal disc overgrowth. Because of its phenotype that follows homozygous loss, we consider *wts* to be a tumor suppressor gene. The *wts* gene is defined by the breakpoints of overlapping deficiencies in bands 100B1,2, and by lethal P-element insertions and excisions. It encodes a protein kinase that is very similar to the DM-PK. Although myotonic dystrophy is defined by a neuromuscular disorder, it can be associated with pilomatrixomas, which are otherwise rare epithelial tumors, and with other tumors including neurofibromas and parathyroid adenomas. Our results raise the possibility that homozygous loss of the DM-PK may contribute to the development of these tumors.

S03-27

DETECTION OF MINIMAL RESIDUAL DESEASE BY FLUORESCENT IN SITU HYBRIDIZATION (FISH) IN MANTLER CELL LYMPHOMAS.

Soldati G., Zucca E., and Cavalli F.
Dept. of Oncology, "La Carità" Hospital, 6600 Locarno.

Introduction. The most consistent chromosomal aberration of mantle cell lymphoma (MCL) is the translocation t(11;14)(q13;q32). As a result the bcl-1 locus on chromosome 11 is juxtaposed to the immunoglobulin heavy chain gene located on chromosome 14. The subsequent deregulation of the bcl-1 gene, may perpetuate the G1-S cell cycle transition leading to the lymphoma development.

Materials. The peripheral blood (PB) of 11 patients and 3 healthy donors was cultured for 3 days with PHA and blocked 30 min with Colcemid. Nuclei were extracted (75mM KCl), fixed in meth/acetic ac. (3:1) and dropped onto glass slides. They were then denatured at 65°C 2min in parallel with biotinylated DNA probes for chr.11 (whole paints) and hybridized overnight at 42°C. Hybridizations on metaphases and on interphase nuclei were detected by FITC-Avidin and biotinylated anti-Avidin and quantified by means of an image analysis system (CCD-Photometrics) under a fluorescent microscope.

Results. In the PB of healthy donors we always found less than 2% of nuclei bearing the t(11;14) split signal, therefore we decided to use a diagnostic cut-off level of 5%. Three out of seven patients in clinical remission showed positivity for t(11;14).

Discussion. The feasibility of interphase FISH for the detection of t(11;14) has proved to be useful compared to classic cytogenetics also in patients with no evidence of disease. The main advantage of such a technique is the possibility to detect the t(11;14) in interphase nnuclei that greatly enhances its sensitivity. Furthermore, the use of a digital image analisys system improves the background/signal ratio and could lower the cut-off level to less than 5%.

S03-28

The SRP14 subunit of the signal recognition particle occurs in multiple complexes in primate cells suggesting novel roles for SRP9/14

Fabrice Bovia & Katharina Strub, Département de Biologie Cellulaire, Université de Genève, CH-1211 Genève 4

Translocation of proteins into the endoplasmic reticulum requires the signal recognition particle (SRP), a ribonucleoprotein composed of one RNA molecule and six polypeptides. The heterodimeric subunit SRP9/14 is implicated in the translational control function of SRP. We have studied the regulation of SRP biogenesis *in vivo* with polypeptide-specific antibodies against SRP subunits. We observed an interesting size variation of SRP14 in different mammalian cells. A SRP14 cDNA isolated from a human cell line is highly homologous to murine SRP14 but contains an additional alanine/threonine-rich region at the C-terminus. This region results from the translation of trinucleotide repeats and accounts for the size difference between human and murine SRP14 proteins. Previously, such repeats have been shown to cause neurological disorders due to the expansion of the repetitive sequence. Further, we noticed that SRP9/14 accumulates in excess over other SRP subunits in primate but not in rodent cells. Cellular fractionation experiments demonstrated that SRP9/14 exists in at least three distinguishable complexes: (i) bound to SRP; (ii) in a 8.5S particle bound to small cytoplasmic Alu RNAs and (iii) as a cytoplasmic complex not stably associated with distinct structures. This together with the species-specific regulation of SRP9/14 expression suggests that the heterodimer may play additional roles in the translational control of gene expression and/or Alu transcript metabolism.

S03-29

ECTOPIC EXPRESSION OF THE AXON-ASSOCIATED CELL ADHESION MOLECULE AXONIN-1 IN MOTONEURONS, USING A DEFECTIVE RECOMBINANT ADENOVIRUS.

Giger, R. J., Ziegler, U., *Hermes, W.T.J.M.C., Schrimpf, S., Vogt, L., *Verhaagen, J. and Sonderegger, P. Biochemisches Institut der Universität Zürich, 8057 Irchel.
*Netherlands Institute for Brain Research, Amsterdam, NL.

The ability to manipulate the expression of genes in neurons, provides new opportunities to study their function. Vectors based upon DNA viruses such as recombinant herpes simplex type 1, adeno-associated virus, and adenovirus can be used *in vitro* and *in vivo* for gene transfer into a variety of different cells, including postmitotic cells such as neurons. By homologous recombination we constructed a defective adenovirus, containing the coding region of the axon-associated cell adhesion molecule axonin-1 under the transcriptional control of the cytomegalovirus promoter. Infection of motoneurons dissected from the ventral horn of chicken embryonic day 10 spinal cord resulted in ectopic expression of axonin-1. Recombinant axonin-1 exhibited the same subcellular distribution as native axonin-1. Thus, viral vectors provide potent tools for studying the function of neuronal cell adhesion molecules in axonal growth and regeneration.

S03-30

EXPRESSION OF A TRANSFORMING GROWTH FACTOR-β1 TRANSGENE IN MURINE β CELLS ALTERS THE STRUCTURE AND RELATIVE PROPORTION OF ENDOCRINE AND EXOCRINE PANCREAS

Nichols, A., Sanvito, F., Herrera, P., Huarte, J., Wohlwend, A., Vassalli, J.-D., and Orci, L.
Dep. of Morphology, C.M.U., CH-1211 Geneva 4.

TGF-β1 is a multipotent cytokine involved in a variety of physiological, pathological and developmental processes. We have previously demonstrated that it modulates the differentiation of the exocrine and endocrine portions of the developing pancreas in organocultures. To further investigate this observed effect, we generated mice bearing a mutated murine TGF-β1 transgene controlled by the rat insulin II gene promoter. Expression of this transgene leads to a conspicuous disorganization of islet architecture caused by a massive fibrosis; however, the mice are normoglycemic. The exocrine portion of the gland is drastically reduced in aged mice. These results demonstrate the selective effect of TGF-β1 on the different portions of the pancreas, and raise the possibility of a role for this cytokine in controlling the relative proportion of the exocrine and endocrine components.

S03-31

AN EVALUATION OF NEEDLE BIOPSIES IN THE INVESTIGATION OF MUSCLE DISORDERS AND FOR OBTAINING HUMAN SATELLITE CELLS

M.R. Magistris, A. Baroffio, G.-P. Pizzolato, M.A. Morris, C.-R. Bader, C. Gabay, M. Estade. Réseau Neuro-Musculaire, Centre Médical Universitaire et Hôpital Cantonal Universitaire, 1211 Genève 4.

The surgical muscle biopsies commonly used for the diagnosis of neuromuscular diseases are time-consuming and require anaesthesia in children. Needle muscle biopsies do not have these drawbacks but their use has been limited, allegedly because of inadequate size of specimens. We have evaluated a method developed at Tufts University [Coté et al. (1992) Neurology 42:2212]. The technique uses a 14-gauge needle designed for prostate biopsies and an automatic device that propels first a core needle with a sampling notch, and then a tubular knife which cuts the muscle, leaving a small sample in the notch (10-40 mg, representing up to 500 muscle fibres). The two steps last less than 100 ms. After local skin anaesthesia, up to four samples can be collected from one site. The samples have been used for light and electron microscopy, molecular genetic analysis, and the extraction of satellite cells for cultures. The latter are recovered by dissociating the sample and collecting individual satellite cells with a micropipette. During the past 10 months we have examined 55 patients (age: 7-80 years), collecting 144 samples in 59 muscles. The technique is well tolerated by the patients, who never declined multiple samplings. Needle biopsies can be more easily performed and repeated than surgical biopsies. It is now our method of choice for the majority of diagnostic muscle biopsies.

S03-32

ACETYLCHOLINE RECEPTOR ACTIVATION INCREASES FUSION OF HUMAN MUSCLE SATELLITE CELLS.

L. Bernheim, R.M. Krause, M. Hamann, A. Baroffio, J.-H. Liu, J.P. Ternaux, and C.R. Bader. Département de Physiologie and Division de Recherche Clinique Neuromusculaire, CMU, 1211 Geneva 4, Switzerland.

Fusion of myogenic cells is important for muscle growth and repair. A possible involvement of nicotinic acetylcholine receptors (nAChR) in the fusion process of myoblasts derived from human satellite cells was examined in this study. First, using whole-cell and single-channel voltage-clamp recordings, we investigated the expression of nAChR in freshly-isolated satellite cells, in cultured proliferating myoblasts, in myoblasts just before their fusion into myotubes, and in myotubes. Second, we evaluated the effect of nicotinic agonists and antagonists on the rate of myoblast fusion in culture. And third, looking for possible sources of ACh, we examined (using chemiluminescent method) whether endogenous ACh was present in myogenic cells.

Our results suggest that i) nAChR expression increases drastically just before myoblast fusion into myotubes, ii) nAChR activation increases the rate of myoblast fusion, and iii) ACh is synthesized and released by myoblasts and myotubes. We hypothesize that nAChR activated by ACh secreted by myoblasts and myotubes provides a positive feed-back mechanism which accelerates the process of myoblast fusion and therefore improves muscle regeneration.

S03-33

THE SEGREGATION OF NEW HUMAN MUSCLE SATELLITE CELLS OCCURS AS MYOBLASTS PROLIFERATE

M. Hamann, A. Baroffio, and C.R. Bader, Département de Physiologie, Centre Médical Universitaire and Division de Recherche Clinique Neuro-Musculaire, Hôpital Cantonal Universitaire, 1211 Genève 4.

Muscle satellite cells (SC) are myoblasts that are normally quiescent, and which, when stimulated, contribute to muscle repair. In vitro, after proliferation, myoblasts can be induced to fuse by exposure to a differentiation medium (DM). However, approximately 30% of the myoblasts fail to fuse in DM. We call them non-fusing myoblasts (NFMB). We recently demonstrated that NFMB are phenotypically and developmentally similar to quiescent SC, suggesting that SC have the ability of self-renewal in vitro. In the present study we examined whether NFMB are segregated before or after exposure to DM. We found that the progeny of a single SC proliferating in vitro is made up of two populations of cells differing by the size, the rate of proliferation, the expression of α-SR actin and the self-renewal potential. The larger cells proliferate, as shown by ^{3}H-thymidine incorporation, and 54±7% express α-SR actin. On the other hand, the smaller cells do not divide and remain undifferentiated. When subcloned, i.e. isolated from the rest of the clonal progeny and cultivated as single cells, 60% of both small and large cells survive. However, only 6±5% of the larger cells resume proliferation, none giving rise to a myogenic progeny, whereas 43±11% of the smaller cells resume proliferation, 7% being self-renewing cells, giving rise to a myogenic progeny. These observations suggest that future SC are segregated under culture conditions promoting myoblasts proliferation.

S03-34

Disruption of the γ2 Subunit Gene of $GABA_A$ Receptors Resulting in Benzodiazepine Insensitive Mice and Neonatal Lethality

Uwe Günther[1], Jack Benson[1], Dietmar Benke[1], Jean-Marc Fritschy[1], Guadalupe Reyes[1], Frederic Knoflach[1], Adriano Aguzzi[2], Florence Crestani[1], Michele Arigoni[1], Yolande Lang[3], Horst Blüthmann[3], Hanns Möhler[1] and Bernhard Lüscher[1]. [1]Pharmakologisches Institut, Universität Zürich, 8057 Zürich, [2]Institut für Neuropathologie, Universitätsspital, 8091 Zürich, Switzerland, [3]PRTB, Hoffmann-LaRoche, 4002 Basel.

CNS states such as alertness, anxiety, and memory can be modulated by drugs acting at the benzodiazepine (BZ) binding site of hetero-oligomeric γ-aminobutyric acid type A ($GABA_A$) receptors. The BZ site has been conserved during evolution of the animal kingdom but its function under physiological conditions is as yet unknown. Using targeted disruption of the γ_2 subunit gene of $GABA_A$ receptors, we produced mice largely devoid of BZ binding sites but containing almost normal numbers of $GABA_A$ receptors. Patch clamp analysis of dorsal root ganglia neurons revealed GABA-induced currents that were normally inhibited by bicuculline and picrotoxin and potentiated by pentobarbital but which were insensitive to flunitrazepam. In addition, diazepam was inactive behaviorally. Thus the γ_2 subunit is not essential for the assembly of the remaining subunits into functional GABA-gated channels, but is a prerequisite for the formation of BZ sites in vivo. Minor reductions in GABA sensitivity and chloride conductance were also apparent in γ_2 subunit-deficient $GABA_A$-receptors. Possibly as a consequence of altered channel properties and/or due to the lack of receptor regulation by endogenous BZ ligands, mice lacking γ_2 subunits were cyanotic and hyperactive at birth, became severely growth retarded and died before 17 days of age. Thus, the γ2 subunit is dispensable for embryonic development but stringently required for postnatal development.

S03-35

GENE THERAPY IN CYSTIC FIBROSIS: PRE-CLINICAL AND CLINICAL STUDIES WITH ADENOVIRUS VECTORS. Rochat T., Division de Pneumologie, Hôpital Cantonal Universitaire, Genève.

In 1989, the identification of the gene for the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) opened the way for gene therapy in cystic fibrosis (CF). CFTR is a cAMP-activated chloride channel at the apical membrane of epithelial cells. Patients with CF usually die from respiratory failure consecutive to abnormal secretions and recurrent infections in the bronchi, due to the dysfunctioning of CFTR. Several groups have reported that the most efficient vectors so far, to introduce exogeneous CFTR cDNA into the bronchial epithelial cells, are recombinant adenoviruses (Ad-CFTR). Expression of mRNA and protein from the transgene has been achieved with success in cultured epithelial cells, in animals, and in CF patients. Correction of the abnormal, epithelial electrophysiology has been obtained *in vitro*, and studies are in progress *in vivo*. Regarding safety issues, Ad-CFTR are made replication-deficient by deletion of early genes, thus reducing their pathogenic potential and spreading. They do not integrate in the cell genome, which minimizes the risk of mutagenesis. However, a dose-dependent inflammatory reaction has been observed after instillation of Ad-CFTR in the bronchi. Construction of second generation viruses will attempt to reduce side effects, while increasing the efficiency of gene transfer.

S03-36

RB-1 AND P53 TUMOUR SUPPRESSOR GENE FUNCTION AND CELL DEATH.

A.R. Clarke. CRC Laboratories, Edinburgh, UK.

We have generated two murine strains, deficient in the tumour suppressor genes p53 and Rb-1. Animals homozygous for a disruption of the Rb-1 gene die *in utero*, characterised by abnormal patterns of proliferation and programmed cell death (apoptosis). Animals heterozygous for this deficiency survive to birth, but develop tumours of the intermediate lobe of the pituitary.

Mice homozygous for a the p53 deletion survive to birth, but then succumb to a variety of malignancies, predominantly of the thymic lymphoid lineage. Heterozygotes develop malignancies at a slower rate and with a different spectrum. To further investigate the relationship between p53 dysfunction and malignancy we have analysed the role of this gene in the induction of apoptosis. In the cell systems we have investigated to date, namely thymocytes, enterocytes and IL-7 dependent pre B cells we have found p53 to be crucial for the induction of apoptosis after exposure to DNA damaging agents. We therefore have evidence that p53 deficient cells are more likely to persist following DNA damage, with an increased potential to become malignant.

Signal transduction in plants and plant biology

S04-01

PHOSPHORYLATION OF A PLASMA MEMBRANE PROTEIN IN RESPONSE TO OLIGOGALACTURONIDE DEFENSE SIGNALS.

P. Reymond, S. Grünberger, K. Paul and E. E. Farmer; Institut de Biologie et de Physiologie végétales, UNIL, 1015 Lausanne.

Oligogalacturonides are plant cell wall-derived regulatory molecules which trigger numerous defense responses. *In vitro*, a polygalacturonate-rich fraction of tomato leaf cells enhances the phosphorylation of a 34 kD protein (pp34) in plasma membranes purified from potato, tomato and a more distantly related dicotyledon, soybean. Purified oligogalacturonides of 13 to at least 26 residues long also stimulate pp34 phosphorylation. This stimulation pattern differs from the induction of many known defense responses *in vivo* where a narrower range of smaller fragments of 10 to 15 residues are active. Based on these differences we suggest that observed effects of applied oligogalacturonides on defense responses may not necessarily reflect the situation during pathogenesis since the cell wall could act as a barrier to many active oligo- and polygalacturonides. The correlation between pp34 phosphorylation and defense gene expression is under investigation as are efforts to clone a pp34 cDNA.

S04-02

A CHANGED INTERPLAY OF PROTEIN KINASES AND PROTEIN PHOSPHATASES IN THE ELICITOR RESPONSE

G. Felix, Friedrich Miescher-Institut, Basel

Suspension cultured tomato cells respond to a variety of fungal and bacterial compounds, "elicitors", with rapid alkalinization of their culture medium and increased biosynthesis of the plant stress hormone ethylene. The elicitor response is paralleled by changes in the phosphorylation of specific proteins, is blocked by protein kinase inhibitors like K-252a and can be mimicked by the protein phosphatase inhibitor calyculin A. Both types of drugs affect the induction process within less than 1 min, indicating a dynamic balance of protein phosphorylation and dephosphorylation in the regulation of the elicited state. It remains open whether transduction of the elicitor signal is primarily based on reduced activity of a protein phosphatase or on increased activity of a protein kinase.

S04-03

SIGNALS INVOLVED IN PLANT DEVELOPMENT.

Fischer Ch., Neuhaus G., Institute for Plant Science (Dept. of Prof. I. Potrykus), ETH - Zürich, Universitätstrasse 2, CH - 8092 Zürich, Switzerland.

Our aim is to investigate the mechanisms underlying the attainment of the bilateral symmetry from the axial symmetry during early monocot embryogenesis. For this purpose, *in vitro* culture conditions for globular to early transition wheat (*Triticum aestivum* L.) embryos have been investigated. This culture system allows up to 70 % of the cultured embryos to develop through normal direct embryogenesis. Using this culture system, we studied the influence of auxin on the shift from the radial to the bilateral symmetry during early embryogenesis. Auxins (2,4,5-trichlorophenoxyacetic acid, 2,4 dichlorophenoxyacetic acid) as well as an auxin transport inhibitor (2,3,5-triiodobenzoic acid) added directly to the culture medium, affect the embryonic symmetry, the scutellum and the shoot apical meristem differentiation. The phenotypes obtained are dependant on the concentration used and on the developmental stage of the isolated embryos. By contrast, a compound assumed to be an auxin antagonist (2-(p-chlorophenoxy)-2 methylpropionic acid) has no visible effect on the embryonic symmetry. These observations indicate that auxin might influence the change from the radial symmetry to the embryonic polarity during monocot embryogenesis.

S04-04

ELICITOR RECOGNITION AND INTRACELLULAR SIGNAL TRANSDUCTION IN PLANT DEFENCE.

Nürnberger, T., Nennstiel, D., Jabs, T., Sacks, W., Hahlbrock, K., Scheel, D. Max-Planck-Institut für Züchtungsforschung Köln.

An oligopeptide of 13 amino acids identified within a 42 kDa glycoprotein elicitor from Phytophthora megasperma was shown to be necessary and sufficient to stimulate a complex defence response in parsley cells comprising H^+/Ca^{2+} influxes, K^+/C^- effluxes, an oxidative burst, defense-related gene activation and phytoalexin formation. Binding of ^{125}I-Pep-13 to parsley membranes was specific, reversible, and saturable. Identical structural features of Pep-13 were found to be responsible for binding and initiation of all plant responses analyzed. A 91-kDa parsley plasma membrane protein was identified to be the receptor mediating activation of a multi-component defence response.

S04-05

AUXIN SIGNALLING AND ACTION AT THE PLASMA MEMBRANE OF PLANT CELLS

H. Barbier-Brygoo, N. Leblanc, C. Perrot-Rechenmann, J-M. Pradier, S. Thomine, S. Zimmermann and J. Guern. Institut des Sciences Végétales, CNRS, F-91198 Gif-sur-Yvette Cedex, France.

The study of auxin-induced modifications of the electrical potential of tobacco mesophyll protoplasts supported a model where a soluble auxin-binding protein would interact with a docking transmembrane protein to constitute functional auxin receptors at the plasma membrane. Work is in progress to identify these two components, the auxin binding unit (Nt-abp) and the transmembrane protein. To characterize the basis of the electrical response of protoplasts to auxin, ion channels at the plasma membrane of protoplasts from tobacco cell suspensions were studied by the patch-clamp technique. An anion current was described which showed a phosphorylation-dependent voltage regulation. The active auxin 1-NAA and the auxin agonist antibody D16 caused a time- and concentration-dependent shift of the activation potential of the channel. Present investigations aim at establishing if these early membrane responses to auxin participate in other hormonal responses such as regulation of gene expression.

S04-06

CELL SIGNALLING THROUGH CYTOSOLIC CALCIUM.

Anthony Trewavas. Institute of Cell and Molecular Biology, University of Edinburgh, Edinburgh EH9 3JH, Scotland.

Plant cells receive a constant stream of environmental information to which they respond. Many of these signals impinge upon a cellular reaction network of considerable complexity and regulation which is constructed around calcium. Calcium enters the cytoplasm through channels clustered to varying degrees in the plasma membrane and some organelle membranes; channels may also direct calcium into intraorganelle spaces such as the nucleus. There is fine grain differentiation of free calcium concentrations particularly in membrane microdomains and these change in unknown ways during signalling.
Understanding aspects of this complex control system requires knowledge of the cellular distribution and movement of calcium-sensitive enzymes and proteins and the local distribution of intracellular calcium itself. The Molecular Signalling Group at Edinburgh has developed a novel method with which the complexity of distribution of calcium can be approached. Plants have been transformed with the cDNA for the calcium sensitive luminescent protein aequorin; luminous plants have been generated whose luminosity measures intracellular calcium. This technique will be described and its future indicated. The information so far gained concerning mechanical signalling, oxidative stress and targeted aequorins will be outlined.

S04-07

ACCUMULATION OF TRANSCRIPTS SPECIFIC FOR GENES ENCODING ENZYMES OF THE PRECHORISMATE PATHWAY IN ELICITOR TREATED TOMATO CELLS.

J. Schmid[1], J. Görlach[1], H.-R. Raesecke[1], T. Boller[2] & N. Amrhein[1]
[1]Institute of Plant Sciences, ETH-Zürich, CH-8092 Zürich; [2]Friedrich-Miescher Institute, P.O. Box 2543, CH-4002 Basel.

We have studied transcript levels of six genes (i.e. two 3-deoxy-D-*arabino*-heptulosonate 7-phosphate synthase genes, one shikimate kinase gene, one 5-*enol*pyruvylshikimate 3-phosphate synthase gene, and two chorismate synthase genes) corresponding to four steps of the prechorismate pathway, in cultured tomato cells exposed to fungal elicitors. The abundance of transcripts specific for some of these genes increased 10-20 fold within 6 hours after elicitor treatment as did the abundance of phenylalanine ammonia-lyase-specific transcripts and the synthesis of ethylene.

S04-08

ISOLATION AND MAPPING OF GENOMIC CLONES ENCODING SOYBEAN ISOCITRATE LYASE AND MALATE DEHYDROGENASE.

Guex, N., Richter, H., Schick, B., and Widmer, F. Institut de Biologie et de Physiologie Végétales de l'Université, CH-1015 Lausanne.

DIG labelled probes synthesized from glyoxysomal malate dehydrogenase (gMDH; EC 1.1.1.37) and isocitrate lyase (ICL; EC 4.1.3.1) cDNA clones were used for screening a λ-GEM 11 genomic library constructed from soybean (*Glycine max.* L. cv. Maple arrow) hypocotyl isolated DNA. Three gMDH clones and one ICL clone were purified. The three gMDH clones appear to be siblings with an insert of about 14.5 kb. Preliminary mapping data suggest the absence of any introns, or the occurrence of very small ones only. This is interesting as compared to the mouse mitochondrial and cytoplasmic MDH isoforms, which possess 8 introns between 0.3 and 4.8 kb (Setoyama et al. *J. Mol. Biol.* (1988) **202**, 355-364). Closer analysis of the gMDH genomic clone will allow to compare the gene organizations and protein sequences of various MDH isoforms. Evolutionary relationships could thus be postulated. The ICL genomic clone has an insert of approximately 22 kb and mapping results suggest that the whole gene is present. Subcloning of these genes is in progress, with the purpose of isolating their promotors and thereby to study their regulation during germination and senescence.

S04-09

PHOSPHORYLATION OF LIPIDS IN SPINACH CHLOROPLAST ENVELOPES

Müller, M.O., Ruffieux, L., Bovet, L. and Siegenthaler, P.A.
Laboratoire de Physiologie Végétale de l'Université CH-2007 Neuchâtel

In the presence of [γ^{32}P]ATP spinach chloroplast envelope membranes are able to phosphorylate in vitro several of its proteins (Siegenthaler and Bovet, Planta 190: 231-240, 1993). Simultaneously, other components extractable in chloroform-methanol (1:2) were also found to be ^{32}P-labelled. The purpose of this investigation was to identify these radioactive compounds. TLC patterns revealed that some of them exhibited same Rfs as phosphatidic acid (PA) and its lyso-derivative (L-PA) as well as phosphatidylinositol-4-phosphate (PIP) and phosphatidylinositol-4,5-phosphate (PIP_2). The identity of PA and L-PA were verified by mild deacylation procedure. Labelled PA and L-PA were found to be located only in the inner envelope membrane fraction whilst labelled compounds, likely to be PIP and PIP_2, were associated essentially with the outer envelope membrane one. In order to determine whether a diacylglycerol kinase (DAG kinase) was involved in generating PA in the chloroplast envelope, immunoblots were carried out with anti-DAG kinase antibodies. Results showed that an antiserum, raised against a prokaryotic DAG kinase, recognized a protein of about 19 kDa in the chloroplast envelope. This protein was located in the inner membrane of the envelope and partitioned into the organic phase following Triton X-114 two-phase separation. These results will be discussed in terms of the possible role of phosphorylated acyl lipids and DAG kinase in chloroplast envelope membranes.

Supported by the Swiss National Science Foundation Nr. 3100.33693.92.

S04-10

PURIFICATION OF MITOCHONDRIAL AND GLYOXYSOMAL CITRATE SYNTHASE ISOFORMS FROM CRUDE EXTRACTS OF SOYBEAN COTYLEDONS

Escher C.-L., Richter H., Widmer F., Institut de Biologie et de Physiologie Végétales de l'Université, CH-1015 Lausanne

Soybean cotyledons (*Glycine max.* L.) possess two forms of citrate synthase (CS; EC 4.1.3.7) : a mitochondrial form (mCS) active in the Krebs cycle and a glyoxysomal form (gCS) active in the glyoxylate cycle. mCS is constitutively expressed, whereas gCS is induced at germination, disappears when fat reserves are depleted, and is apparently reinduced when the cotyledon is senescent. Both forms of CS show many similarities and cannot be easily distinguished. Antibodies or nucleotidic probes would permit to study the mechanism responsible for the regulation of gCS expression.
While CS isoforms can be separated from isolated organelles, the method has shown poor recovery yields. CS isoforms are thus purified from crude extracts. The first purification step is a gel filtration that separates the two forms using different salt concentrations, since only gCS aggregates in low salt buffer. gCS is then deaggregated by high salt buffer gel filtration. Successive chromatography and finally SDS-PAGE permit to isolate three mitochondrial and two glyoxysomal isoforms. These isoforms are analysed using two dimensional electrophoresis, and then used to raise antibodies or microsequenced to design nucleotidic probes.

S04-11

RESISTANCE TO NEPOVIRUSES IN GRAPEVINE: EXPRESSION OF SEVERAL PUTATIVE RESISTANCE GENES IN TRANSGENIC PLANTS.

[1]Marc-Martin S., [2]Ramel M.-H., [2]Gugerli P., [1]Krastanova S. and [1]Spielmann A.
[1]Université de Neuchâtel, Laboratoire de biochimie végétale, Emile-Argand 11, CH-2007 Neuchâtel; [2]Station fédérale de recherches agronomiques, Changins, Département de virologie, Ch-1260 Nyon

The most important soil-borne grapevine viruses are transmitted from the roots of infected vines to the neighbouring healthy ones by nematodes (nepoviruses). Three such viruses are known to occur in Swiss vineyards: grapevine fanleaf virus (GFLV), arabis mosaic virus (ArMV) and raspberry ringspot virus (RRSV). The nepoviruses cause quick destruction of young plants or a gradual decline over several years (infectious degeneration). GFLV is the most widespread virus in grapevines and is the nepovirus of greatest economic importance.
Several chimeric genes comprising either the GFLV coat protein (CP), the ArMV coat protein, the GFLV putative replicase, or a human oligoadenylate synthetase gene were constructed under a constitutive promoter (CaMV 35S or the soybean *tef*S1).
Transgenic plants containing one of the the various chimeric genes described above were produced by cocultivation of *N. benthamiana* leaf discs or grapevine somatic embyogenic calli with *A. tumefaciens* LBA4404 harboring the different binary vectors.
Expression and possible protection effect of the various transgenes will be presented.

S04-12

Promoter Analysis of Marker Genes for Specific Expression Patterns in the Apical Meristem

Fleming A. J., Canavescini S. and Kuhlemeier C.
Institute of Plant Physiology, Bern University

Our earlier work showed that mRNAs encoding a lipid transfer protein (ltp) and Rubisco small sub-unit (rbcS) are expressed in specific spatial patterns in the apical meristem. RT.PCR analysis and fluorescent imaging of promoter-reporter gene constructs in transgenes revealed a hierarchy of rbcS gene transcription during early leaf development. Analysis of an ltp promoter revealed a complex pattern in the meristem and, unexpectedly, expression in root hairs. These results show how apparently simple transcript patterns may reflect complex spatial/temporal combinations of promoter activity.

S04-13

PHOSPHORYLATION OF EUKARYOTIC INITIATION FACTOR 4A (eIF-4A) IN *NICOTIANA TABACUM*

Roel Op den Camp and Cris Kuhlemeier
Institute of Plant Physiology, University of Berne

Eukaryotic translation initiation is a complex process requiring a large number of proteins. One of these proteins, eIF-4A, is thought to act as an ATP-dependent RNA-helicase in conjunction with eIF-4B and to be a part of the cap binding complex eIF-4F. In rabbit reticulocytes, mouse and yeast two closely related eIF-4A genes are expressed. In maize root tips two isoforms of eIF-4A have been found, one of which appeared to be phoshorylated. The phosphorylation of maize root tip eIF-4A was hypoxia-induced [1]. This suggests a regulatory function for eIF-4A phosphorylation. In *Nicotiana* at least 13 members of eIF-4A have been identified [2,3]. All genes examined showed more or less coordinated expression, with the exception of NeIF-4A8, which is pollen specific expressed [4]. In pollen at least three members of eIF-4A are present. We show that in *Nicotiana tabacum* pollen eIF-4A is also phosphorylated.

1) C. Webster, R. Gaut, K. Browning, J. Ravel,J. Roberts (1991). J.Biol.Chem., 266 (34), 23341-23346
2) G. Owttrim, S. Hofmann, C. Kuhlemeier (1991) Nucl.Acids Res., 19 (20), 5491-5496
3) G. Owttrim, T. Mandel, H. Trachsel, A. Thomas, C. Kuhlemeier (1994). Plant. Mol. Bio. Submitted.
4) K. Brander, C. Kuhlemeier (1995), Plant Mol. Bio., Submitted.

S04-14

SYSTEMIC ACQUIRED RESISTANCE IN POTATO

J.-L. Coquoz, T. Palladino, M. Schneider, A.J. Buchala & J.-P. Métraux
Institut de Biologie végétale, Université, CH-1700 Fribourg, Switzerland

Treatment of the lower leaves of potato plants (*S. tuberosum* L. cv. Bintje with no known resistance genes) with arachidonic acid conferred resistance to the blight pathogens *Phytophthora infestans* and *Alternaria solani* at the upper leaves (systemic protection). Salicylic acid has been proposed to act as a signalling molecule in systemic acquired resistance (SAR) but accumulation of this phenol, in either free or conjugated forms, was observed in only the lower leaves. This was accompanied by the synthesis of the pathogenesis related protein PR1. The local synthesis of the latter could also be induced by treatment with salicylic acid. Accumulation of gene transcripts encoding other PR proteins (chitinase and β-glucanase) were only observed in the lower leaves. Jasmonic acid, another putative signalling molecule, does not appear to be involved in the signal tranduction pathway for SAR in potato.

S04-15

NUCLEOTIDE SEQUENCES OF cDNA CLONES ENCODING THE TWO VACUOLAR PROTON PUMPS FROM MAIZE

Perotti, E., Fraichard, A., Gavin, O., Testuz, J. and Chanson, A., Institute of Plant Biology and Physiology, University of Lausanne, Biology Building, CH-1015 Lausanne

Membrane transport of proton plays a fundamental role in growth and developmental processes in higher plant cells. Two proton pumps, a vacuolar-type ATPase (V-ATPase EC 3.6.1.3) and a pyrophosphatase (V-PPase EC 3.6.1.1), catalyze electrogenic proton translocation across the tonoplast, giving rise to a proton motive force used to transport various molecules into and out of the vacuole. Using PCR and a cDNA library from *Rubus hispidus*, we amplified and sequenced a ~0.5 kb DNA fragment. This DNA fragment was labeled with digoxygenin and used to screen a maize cDNA library (Uni-ZAP XR vector from Stratagene). A ~2 kb cDNA clone was found and sequenced. This clone is highly homologous to the *Arabidopsis thaliana* V-PPase sequence. We also obtained the first sequence of the 70 kDa catalytic subunit of the V-ATPase from maize. A digoxygenin-labeled probe was built using a cDNA clone from carrot (a kind gift from Dr. L. Taiz, University of California, Santa Cruz) using specific primers and a PCR technique. This probe was used to screen a maize cDNA library (Uni-ZAP XR vector). A ~2 kb clone was found and sequenced. This clone is highly homologous to the carrot 70 kDa catalytic subunit. These two clones will be used to build digoxygenin-labeled probes to study the expression of the tonoplast proton pumps of maize seedlings by *in situ* hybridization.

S04-16

Secretion of cell-wall degrading enzymes by *Ophiostoma novo-ulmi* and *Ophiostoma ulmi* grown on different carbon sources

Binz, T. & Canevascini, G., Institut de Biologie végétale, Rue Albert Gockel 3, CH-1700 Fribourg

Dutch elm disease (DED) is a vascular wilt syndrome which has been most destructive in the last 25 years all over the northern hemisphere. It is caused by the ascomycete *Ophiostoma ulmi sensu lato* which is a typical dimorphic fungus. Cell wall degrading enzymes are often involved in vascular wilts of plants but their rôle is mostly circumstantial. Here we report the ability of *O. ulmi sensu lato* to produce polysaccharide degrading enzymes when grown on different carbon substrates. The organism was able to secrete endo-xylanase, polygalacturonase and several glycosidases when it was cultivated in its mycelial form whereas yeast cells only produced limited amounts of β-glucosidase. No clearcut correlation could be found between the aggressiveness of *O. ulmi* (formerly termed non aggressive) and *O. novo-ulmi* (aggressive) when the amount of secreted enzymes was compared. However, when elm sapwood, the natural substrate of both pathogens was used for growth differences in the production of aryl-β-galactosidase became apparent. On this substrate, isolates of the EAN (Eurasian race) subgroup of *O. novo-ulmi* secreted relatively high amounts of polygalacturonase compared to the NAN (North American race) subgroup or *O. ulmi* isolates.

S04-17

THE OCTADECANOID SIGNALLING PATHWAY MEDIATES DEFENCE RESPONSES AGAINST PATHOGEN ATTACK IN RICE

Patrick Schweizer, Antony Buchala and Jean-Pierre Métraux
Institut de biologie végétale, CH-1700 Fribourg

The plant growth regulator jasmonic acid (JA) has been implicated in plant reactions to stresses like wounding and dessiccation, while its potential role in defence against pathogens is still speculative. Exogenously applied JA or its methyl ester (MeJA) induced local accumulation of extracellular pathogenesis-related (PR) proteins in rice, including PR1. PR1 induction by 2,6-dichloroisonicotinic acid (INA), a well established inducer of resistance in plants, was blocked by inhibiting JA biosynthesis. While JA was not sufficient to induce local acquired resistance to *M.grisea*, systemic acquired resistance was expressed in new leaves growing after JA treatment of older leaves. Simultaneous application of INA and JA had an additive effect on protection and resulted in synergistic induction of the accumulation of PR1 and another major, INA-induced protein of unknown function. JA levels tended to increase in leaves of both infected and INA-treated plants. The data suggest a complex interaction between the octadecanoid and another, still unknown,signalling pathway in rice expressing the phenomenon of acquired disease resistance.

S04-18

HEAT-INDUCED DISEASE RESISTANCE IN BARLEY

Laurence Vallélian, J.-P. Métraux & P. Schweizer
Institut de biologie végétale, Université de Fribourg, CH. 1700 Fribourg, Switzerland

In leaves of the susceptible barley cultivar "Golden Promise", a heat treatment (HT) of 40 to 60 seconds in water at 50°C induced local and transient resitance to the powdery mildew *Erysiphe graminis* f.sp. *hordei*. The mechanism of protection was neither dependent on papilla formation which may prevent penetration by *E. graminis* nor on a hypersensitive response (HR).
Protection was correlated with enhanced resistance of heat-treated leaves to protoplasting enzymes and induction of a pattern of mRNAs. One heat-induced mRNA has been shown to be also induced by the pathogen. In contrast, heat-induced resistance was not correlated with induction of pathogenesis-related proteins, peroxidase, HSP 90 or accumulation of salicylic acid. Nevertheless, an unknown protein of 22kD disappeared from the intercellular wash fluid of heat-treated leaves.
Work is in progress to purify this protein and to clone heat-induced mRNAs using the new method of mRNA differential display.

S04-19

SALICYLIC ACID AND ITS MODE OF ACTION DURING SYSTEMIC ACQUIRED RESISTANCE IN *ARABIDOPSIS THALIANA*

K. Summermatter & J.-P. Métraux. Institut de Biologie végétale, Université de Fribourg, 1700 Fribourg

Attack of plants by necrotizing pathogens leads to local and systemic acquired resistance against the same or other pathogens even in tissues remotely located from the site of initial attack. We have used *Arabidopsis thaliana* to investigate the mode of action of salicylic acid (SA). Inoculation with *Pseudomonas syringae* on the lower leaf of *A. thaliana* triggers the induction of SAR which correlates with a local and systemic increase in SA. A similar correlation was found for chitinase and peroxidase activities. No changes were observed in catalase activity. A recent hypothesis postulates that SA works by inhibiting catalase activity thus preventing the detoxification of H_2O_2 to O_2, leading to elevated peroxide levels which could induce defence reactions. Our data offer little support for this hypothesis since i) no inhibition of catalase could be observed in tissue where the endogenous levels of SA were elevated either naturally (after infection) or artificially (by feeding SA to the roots) and ii) no inhibition of catalase activity could be obtained *in vitro* using homogenates of *Arabidopsis* leaves. H_2O_2 could induce the accumulation of SA in leaves, suggesting that the H_2O_2 burst might indeed be an event taking place before the induction of the synthesis of SA during SAR.

S04-20

METABOLISM OF SALICYLIC ACID IN CUCUMBER PLANTS

Meuwly P., Mölders W., Crausaz M. and Métraux J.-P. Institut de Biologie végétale, Université de Fribourg, 1700 Fribourg, Switzerland.

Salicylic acid (SA) seems an essential component in the induction of successful defence responses of plants against pathogens. We studied the biosynthesis and catabolism of SA in infected cucumber plants using HPLC separation coupled to diode-array, fluorescence and radioactivity detectors.

Leaf discs from plants inoculated with tobacco necrosis virus (TNV) or *Pseudomonas lachrymans* were vacuum infiltrated with ^{14}C-benzoic acid (^{14}C-BA), ^{14}C-cinnamic acid (^{14}C-CA) or ^{14}C-phenylalanine (^{14}C-Phe). Alternatively, cotyledons were inoculated with TNV and fed with ^{14}C-BA. The nature of bound SA was also investigated using various hydrolytic enzymes.

For the first time in plants, we show that some ^{14}C-Phe can be incorporated in ^{14}C-SA. We also found high incorporation of ^{14}C-BA in ^{14}C-SA and in another polar phenolic compound. Most of bound SA was cleaved with a β-glucosidase indicating that the preferential sink of free SA in infected cucumber is a SA-*o*-glucoside.

S04-21

BIOSYNTHESIS OF SALICYLATE FROM CHORISMATE IN BACTERIA: IDENTIFICATION AND EXPRESSION OF THE STRUCTURAL GENES FROM *PSEUDOMONAS AERUGINOSA*

C. Reimmann[1], L. Serino[1], H. Baur[2], M. Beyeler[1] and D. Haas[1]
[1]Laboratoire de Biologie Microbienne, Université de Lausanne, CH-1015 Lausanne-Dorigny
[2]INTRON, Laboratory for Molecular Biology, CH-8722 Kaltbrunn

In bacteria, salicylate is a precursor of iron-chelating compounds such as mycobactin and pyochelin and displays siderophore activity itself. We have identified two adjacent chromosomal genes termed *pchB* and *pchA* from *P. aeruginosa* PAO1 which are required for salicylate formation. The expression of both genes is repressed by high iron concentrations. *PchA* encodes a polypeptide of 52 kDa with extensive similarity to chorismate-utilizing enzymes, especially to isochorismate synthase, an enzyme that converts chorismate to isochorismate. PchB, a polypeptide of 11 kDa, has no homology to other known proteins. Expression of these genes in *E. coli*, which is naturally deficient in the formation of salicylate, enables this host to synthesize salicylate. Salicylate formation could also be demonstrated *in vitro*: Incubation of chorismate with crude extracts of *P. aeruginosa* overexpressing PchA and PchB resulted in the formation of equimolar amounts of salicylate and pyruvate. We propose that in *P. aeruginosa* salicylate is formed from chorismate. This pathway differs from those assumed to occur in plants.

S04-22

IMPORT OF PRECURSOR PROTEINS INTO ISOLATED CHLOROPLASTS AND THYLAKOIDS OF CHLAMYDOMONAS REINHARDII

A. Rüfenacht & A. Boschetti, Inst. für Biochemie, Universität Bern

Precursors of five chloroplast proteins from Chlamydomonas reinhardii have been synthesized in vitro from cloned cDNA genes: three proteins of photosystem I, cytochrome c_{552} and plastocyanine. Binding to and import into isolated chloroplasts of Chlamydomonas have been shown. With these precursors we studied also the in vitro processing and the specificity of stromal processing enzymes. Furthermore, we established a protocol for the import of these five proteins into isolated thylakoids of Chlamydomonas reinhardii. All five precursors incorporated in a thermolysin insensitive form in thylakoids, though two of the photosystem I proteins, with somewhat reduced efficency. Further studies about the influence of stromal factors and energy requirement of the import in thylakoids are in progress.

S04-23

EFFECT OF VARIOUS CYTOPLASMATIC FRACTIONS ON PROTEINSYTHESIS IN ISOLATED CHLOROPLASTS OF CHLAMYDOMONAS REINHARDII.

Oliver Schläfli and Arminio Boschetti. Institut für Biochemie, Universität Bern, Freiestr 3 CH-3012.

In chloroplasts isolated from cultures of the unicellular green alga Chlamydomonas reinhardii the incorporation of ^{35}S methionine into several chloroplast proteins, like D_1 (32 kDa protein) and LS (large subunit of ribulose-1,5-bisphosphate carboxylase) could be stimulated by the addition of cytoplasmatic supernatant (S100). This effect was dependent on the time of the cell cycle when the cells have been harvested for the preparation of the S100. Therefore stimulation seems to be the result of a cell cycle dependent cytoplasmatic factor (Blättler et al (1992). The S100 was fractionated by ultrafiltration through cut-off filters, and by reversed phase chromatography. The stimulating activity was found in low molecular weight fractions. Their effects on the in organello protein sythesis and on the polysome pattern will be described.

S04-24

CHOLERA TOXIN ELEVATES PATHOGEN RESISTANCE AND INDUCES DEFENCE REACTIONS IN TRANSGENIC TOBACCO

Beffa, R.S.[1], Neuhaus, G.[2], Meuwly, P.[3], Métraux, J.-P.[3], Meins, F.,Jr.[1] and Nagy, F.[1]
[1]Friedrich Miescher Institute, 4002 Basel, [2]Institut für Pflanzenwissenschaften, ETH, 8092 Zürich, [3] Institut de Biologie Végétale, Université de Fribourg, 1700 Fribourg.

In many plant species the hypersensitive response (HR) at the site of infection triggers systemic acquired resistance (SAR) to subsequent infection with a variety of pathogens. Tissues of transgenic tobacco plants expressing cholera toxin (CTX) mimic SAR state in the absence of infection. They show greatly reduced susceptibility to a bacterial pathogen, *Pseudomonas tabaci*, express a SAR-specific subset of PR proteins, and accumulate high levels of salicylic acid (SAR). Furthermore, using reporter genes, we were able to activate a SAR specific promoter (PR-1) by micro-injecting GTP and CTX. This strongly suggests that the induction of genes expressed during SAR involves the activation of one or more G proteins susceptible to CTX.

S04-25

EXPRESSION AND FUNCTION OF P-GLYCOPROTEINS IN *ARABIDOPSIS THALIANA*

Sidler,M., Graf,S. and Dudler,R., Institut für Pflanzenbiologie, Universität Zürich, 8008 Zürich

The P-glycoproteins (Pgp), which are found in many eucaryotes, are integral membrane proteins that function as energy-dependent transporters. Their substrates belong to a wide array of various substances with different functions. They are involved in several interesting processes, such as the multiple drug resistance (MDR) phenomenon in mammalian tumor cells, signal sequence-independent transport of peptides across membranes or heavy metal detoxification in yeast. Similar processes are also existing in plants, among them herbicide and heavy metal detoxification and signal-molecule transport. To explore the possible role of P-glycoproteins in plants, we set out to analyze the corresponding genes and their products in *Arabidopsis thaliana*. Two of several existing genes were cloned and analyzed (*atpgp1* and *atpgp2*). To investigate the function of ATPGP1 protein we have constructed transgenic plants overexpressing either the sense strand or the anti-sense strand of the *atpgp1* gene and raised an antibody against the protein. Experiments with these plants reveal that the protein may be involved in the development of the plants in connection with hormone action. The transgenic plants also show different resistences against drugs, herbicides and pathogens. The results of these experiments will be presented.

S04-26

MECHANISMS OF INDUCED RESISTANCE IN WHEAT

M.Clausen, A. Kmecl, F. Mauch and R. Dudler, Institut für Pflanzenbiologie, Universität Zürich, Zollikerstr. 107, 8008 Zürich

We are studying induced resistance in wheat using the avirulent barley powdery mildew (*Erysiphe graminis* f.sp *hordei*) as a biological inducer and the virulent wheat powdery mildew (*E.g.* f.sp. *tritici*) as the challenging pathogen. To learn more about the molecular basis of induced resistance, several cDNAs were cloned that correspond to mRNAs which accumulate during the establishment of the resistant state. Apart from the regulation of the respective genes, we are especially interested in the function of the encoded proteins. One of these proteins shows *in vitro* antifungal activity against the wheat pathogen *Fusarium graminearum* and is therefore an attractive canditate for the improvement of wheat disease resistance by genetic engineering. Since induced resistance has common features with horizontal resistance (quantitative resistance), we started to analyze the regulation of the genes corresponding to the cloned cDNAs in wheat lines that differ in their resistance against *E.g.* f.sp. *tritici* but do not contain any known resistance gene for this pathogen. We will also compare the cytological response of resistance-induced wheat with the response of these different wheat lines following powdery mildew infection.

S04-27

CLONING AND EXPRESSION OF cDNAs CODING FOR THE FERREDOXIN:THIOREDOXIN REDUCTASE OF SPINACH

Gaymard, E. and Schürmann, P., Laboratoire de Biochimie végétale, Université, CH-2007 Neuchâtel

Ferredoxin:thioredoxin reductase (FTR) is the key enzyme of the ferredoxin/thioredoxin system, the light-dependent enzyme regulatory system in oxygenic photosynthesis. It is a nucleus encoded iron-sulfur protein, composed of two dissimilar subunits, of about equal size. The catalytic subunit contains a redox-active disulfide bridge functional in the reduction of thioredoxins and a 4Fe-4S cluster. It seems to be rather well conserved between different organisms.
The second subunit is of variable size when comparing the protein from different plant species and has no known catalytic function. We have sequenced both subunits of the spinach FTR on the level of the protein and isolated and characterized full-length cDNAs coding for the subunits. Using PCR we have made a di-cistronic construction carrying, in phase and on the same DNA strand, the coding parts of the genes for both subunits. *E.coli* cells have been transformed with this construction to study the expression of the protein. (SNF 31-37725.93)

S04-28

AMINO ACID SEQUENCE OF SPINACH FERREDOXIN:THIOREDOXIN REDUCTASE CATALYTIC SUBUNIT AND IDENTIFICATION OF THIOL GROUPS CONSTITUTING A REDOX ACTIVE DISULFIDE AND AN Fe-S CLUSTER

Chow, L.-P.[1,2], Iwadate, H.[1], Yano, K.[1], Kamo, M[1], Tsugita, A.[1], Gardet-Salvi, L.[3], Stritt-Etter, A.-L.[3] and Peter Schürmann[3]
[1]Research Institute for Biosciences and [2]Department of Pharmacology, Science University of Tokyo, Japan [3]Laboratoire de Biochimie végétale, Université, CH-2007 Neuchâtel, Switzerland

Ferredoxin:thioredoxin reductase (FTR) a 4Fe-4S protein involved in the light regulation of carbon metabolism in oxygenic photosynthesis catalyses the reduction of thioredoxins with light generated electrons. FTR is composed of two dissimilar subunits, a catalytic subunit and a variable subunit. The catalytic subunit of spinach FTR, which contains the redox active disulfide bridge, was sequenced by conventional protein sequencing techniques and the functional roles of all eight cysteine residues were examined by chemical modifications. The polypeptide chain with a calculated molecular mass of 12,959 daltons consists of 113 amino acids and has a calculated isoelectric point of 5.30. Six of the eight cysteine residues are clustered as Cys-Pro-Cys and Cys-His-Cys groups. Cys19 and Cys27 have no catalytic function, Cys54 and Cys73 (or Cys84) constitute the redox active disulfide bridge of the active site and Cys52, Cys71, Cys82 and Cys84 (or Cys73) are the ligands of the Fe-S cluster. (SNF 31-37725.93)

S04-29

CIS ELEMENTS AND *TRANS*-ACTING FACTORS INVOLVED IN THE INITIATION OF CHLOROPLAST TRANSLATION IN *CHLAMYDOMONAS REINHARDTII*

Otello Stampacchia[1], Jean-Luc Zanasco[1], Bill Zerges[1], Pierre Bennoun* and Jean-David Rochaix[1]
1:Un. of Geneva, Dep. Mol. Biol.;*: IBPC, Paris

The nuclear mutant F15 of *C. reinhardtii* is deficient in translation of the psaB message encoding one of the reaction center polypeptides of photosystem I. This mutation is suppressed by a chloroplast mutation which has been mapped in the 5' untranslated region (5'UTR) of psaB at the end of a Shine Dalgarno-like sequence. Using biolistic transformation and genetic crosses we have introduced chimeric genes consisting of the psaB leader fused to a reporter gene into the chloroplast genome of both wild type and mutant nuclear backgrounds. The chimeric message is translated in the former but not in the latter, indicating that the psaB 5'UTR is the target of the wild-type F15 function.
Similar experiments with the suppressor 5'UTR confirm that the single base change in the suppressor is responsible for the suppression of the F15 mutation. A model will be discussed based on these results.

S04-30

THE 5'UTR OF THE CHLOROPLAST *psb*B mRNA INTERACTS WITH A NUCLEAR FACTOR FOR THE STABILISATION OF THE mRNA

Vaistij F.E., Goldschmidt-Clermont M.& Rochaix J.D.
Department of Molecular Biology, Univ. of Geneva.

The biosynthesis of the photosynthetic apparatus in eukaryotic algae and higher plants depends on the concerted action of the chloroplastic and nuclear genomes. The latter encodes some structural photosynthetic proteins and regulatory proteins for chloroplast gene expression.
The chloroplastic *psb*B gene encodes the P5 polypeptide, a 47 kDa chlorophyll-a binding apoprotein of photosystem II (PSII). The stability of its mRNA is drastically decreased in the nuclear mutant 222E, which is defective in PSII.
In order to understand the mechanisms of stabilisation of *psb*B message, the *psb*B 5'UTR has been fused to the coding region of the *E. coli aad*A gene, which confers spectinomycin resistance when expressed in the chloroplast. We present evidence that the stability of this chimeric mRNA requires wild type 222E function *in vivo*. These results strongly suggest that the *psb*B 5'UTR interacts with a 222E dependent factor to stabilize this mRNA.

S04-31

SITE DIRECTED AND RANDOM MUTAGENESIS OF THE CHLOROPLAST GENE *PSAC* OF *C.REINHARDTII*.

N. Fischer[1], P. Sétif[2] and J.-D. Rochaix[1],
[1] Dep. of Mol. Biol., University of Geneva.
[2] C.E.A Saclay, Paris.

The photosystem I (PSI) is a multiprotein complex which functions to transport electrons from plastocyanin to ferredoxin across the thylakoid membrane using light energy. The 8-9 KD protein encoded by the chloroplast *psaC* gene is the apoprotein of the two 4Fe-4S clusters FA and FB, the terminal electron acceptors.To try to understand the electron flow in this terminal part of PSI, a set of mutations have been created via biolistic transformation: D9N, W31Y/Y81H, E46K, E46Q and Y68F. EPR measurements were made on purified thylakoids from the transformants. The EPR spectra of E46K and W31Y/Y81H show that FB is more reduced in these transformants indicating that these mutations alter the electron flow.
In parallel, a random mutagenesis system by PCR has been developed for the *psaC* gene in which the transformants are screened for the loss of PSI activity and for stable assembly of the PSI complex.

S04-32

Correct folding is important for vacuolar targeting of the tobacco chitinase.

Ernst Freydl, Thomas Boller and Jean-Marc Neuhaus
Botanisches Institut, Abt. Pflanzenphysiologie, Hebelstrasse 1, CH-4056 Basel

The tobacco chitinase A (CHN A) is synthetized as a proprotein CHN A1 which is modified by prolyl hydroxylation (CHN A2) and delivered to the vacuole, where its C-terminal vacuolar targeting peptide (VTP) is cleaved off. When CHN A is expressed transiently in *Nicotiana plumbaginifolia* protoplasts, CHN A2 was also found in the medium. A pulse analysis showed, that two classes of CHN A2 were present in the cells: one class could be immunoprecipitated with anti-VTP and anti-CHN antibodies, the second class only with the anti-CHN antibodies. Coexpression of protein disulfide isomerase (PDI) increased the amount of CHN A synthesized, the amount immunoprecipitated with the anti-VTP antibodies, and also increased the sorting efficiency. Transient expression of the mutant CHN C259A, where the formation of the C-terminal disulfide bond was prevented, showed a strongly decreased intracellular retention. Coexpression of PDI with CHN C259A caused no increased delivery to the vacuole.

S04-33

SIMILARITY OF FUNGAL EXTRADIOL-DIOXYGENASES FROM BETALAIN AND MUSCAFLAVIN PRODUCING SPECIES.

U. Hinz, I. Schmidt & J.P. Zryd, Uni Lausanne, Laboratoire de Phytogénétique cellulaire, Bâtiment de Biologie, 1015 Dorigny

DOPA-dioxygenase is a key enzyme in the biosynthesis of betalains and muscaflavins. Extradiol cleavage of the aromatic ring of DOPA between carbon 4 and 5 gives rise to betalamic acid; cleavage between carbon 2 and 3 gives rise to muscaflavin. The fungus *A. muscaria* (Agaricales) produces both types of pigments; other species (*Amanita* and *Hygrocybe*) produce only musca-flavins.
DOPA-dioxygenase from *A. muscaria* was the first eukaryotic extra-diol ring-cleaving dioxygenase to be purified and cloned. It was shown to produce a mixture of seco-DOPAs, indicating that in this organism the same enzyme was implied in the biosynthesis of betalains and muscaflavins. Western blot analysis showed that this DOPA-dioxygenase was immunologically related to enzymes from muscaflavin producing species, suggesting structural similarities between the fungal dioxygenases. The similarity at the DNA level was studied by Southern blot analysis.

S04-34

TRANSFORMATION OF PLANTS WITH A DOPA-4,5-DIOXYGENASE cDNA FROM *AMANITA MUSCARIA*

L. Müller, U. Hinz and J-P. Zrÿd
Laboratoire de phytogénétique cellulaire, Université de Lausanne, Suisse.

DOPA-4,5-dioxygenase, a key enzyme in betalain biosynthesis, was purified from *A. muscaria*. cDNA clones encoding active DOPA-4,5-dioxygenase were isolated. The recombinant enzyme had a broad substrate specificity similar to the native enzyme, cleaving various *ortho*-diphenols that gave rise to yellow products.
Many plant species produce DOPA or other *ortho*-diphenols, suggesting that the chromogenic activity of DOPA-dioxygenase should be directly detectable *in vivo*. Potential applications include its use as a marker enzyme, or transformation of plants to create new varieties with modified flower coloration.
The DOPA-dioxygenase clone was inserted in a plant expression vector under the control of the 35S promoter to find out whether the enzyme was sufficiently expressed and active in plants.

S04-35

EFFICIENT GENE TARGETING IN THE MOSS PHYSCOMITRELLA PATENS

SCHAEFER D.G. and ZRYD J.-P., Laboratoire de Phytogénétique Cellulaire, Bâtiment de Biologie, UNI Lausanne, 1015 Lausanne Dorigny.

Integrative transformation with non-homologous vectors occurs at a mean relative transformation frequency (RTF) of 0.001 % in the moss Physcomitrella patens. We have previously shown that this frequency increases by one order of magnitude when transgenic strains are retransformed with a vector homologous to the integrated DNA but conferring resistance to another antibiotic, and genetic analysis has demonstrated that this increase is correlated with cosegregation of the markers in the offspring of double resistant transgenic strains (D.Schaefer, PhD thesis, Lausanne 1994).
In order to investigate whether this efficient gene targeting is specific to the presence of artificial loci in the genome, we have performed experiments designed to target single copy natural loci. Internal fragments of 3 independent lambda genomic clones carrying rbcS genes were ligated in transformation vectors and used to transform the wild-type strain. Molecular evidence demonstrating integration of the vectors at high frequency in all three targeted loci will be presented.
Taken together, these data demonstrate that the integration of foreign DNA sequences in the genome of the moss Physcomitrella patens occurs preferentially (ca 90%) at targeted loci by homologous recombination. These findings should lead to the development of a valuable new tool to investigate plant development.

S04-36

CAENORHADITIS ELEGANS HAS AN ABUNDANT PROTEIN C-LEA HOMOLOGOUS TO STRESS PROTEINS IN PLANTS.

Vincent. Bernard, Carine Pythoud, Heinz Tobler, Fritz Müller: Institute of Zoology, University of Fribourg, 1700 Fribourg, Switzerland.

We looked for abundant messengers in *Caenorhabditis elegans* and found a very abundant cDNA by screening a mix-stage cDNA library. The full length 2,300bp cDNA has been sequenced. The transcript is trans-spliced with the splice leader SL1. The deduced amino acid sequence of the *C. elegans* c-LEA gives a protein of 732 amino acids with strong homologies to the plants LEA (late embryogenesis abundant) proteins. It contains 20 repeats of an 11-mer amino acid repeating unit similar to those found in group 3 LEA proteins. In plants their genes are developmentally regulated.

Subsets of LEAs are induced by abscisic acid (ABA), water stress, salt concentration and/or cold stress as well. Functions for LEAs proteins have been suggested to be involved in drought tolerance and embryogenesis but still remain unclear.

To study the expression pattern of c-LEA, antibodies were raised against a fusion protein. We are currently contructing a beta-galactosidase gene construct. These results will be correlated to those found in immunolocalisation. The characterisation of the *C. elegans* homologue of the LEA proteins from plants might be an important step to the understanding of the function of the Lea proteins.

S04-37

GTP bINDING PROTEINS ARE PRESENT ON SPINACH LEAF CELL MEMBRANES.

P-F. Perroud, P. Crespi and H.Greppin.
Lab.Biochimie et Physiologie Végétales.Université de Genève.3, Place de l'Université. 1211 Genève 4.
GTP binding proteins are of major importance in cell signal transduction systems. Implication of this protein type in the transmission of light signals has been shown in different plant cell types.This study shows clearly that GTP binding proteins exist on plasmalemma, Golgi apparatus and on tonoplast of *Spinacia oleracea* leaf cells.Immunoblots show that only an Anti $G_{i}\alpha$-1 regognizes one protein on the Golgi apparatus and an Anti-β subunit, one on both plasmalemma and Golgi apparatus. Preliminary experiments runned on plasmalemma seem to indicate that GTP binding could be partially modulated by red light.

S04-38

Regulation of tissue specific expression of the plant *grp 1.8gene*

Ch. Ringli, S. Torres-Schumann, D. Heierli and B. Keller,
Swiss Federal Research Station for Agronomy, 8046 Zürich

The glycine-rich protein GRP1.8 of *Phaseolus vulgaris* is localised in the primary cell walls of the protoxylem. *vs-1*, a 25 bp sequence of the *grp 1.8* promoter, was found to be necessary for the tissue specific expression of *grp 1.8*. *vs-1* alone confers enhanced and vascular specific expression of the *uidA* (β-glucuro-nidase) gene when cloned 5' upstream of the -82 bp deletion of the 35S CaMV or a -76 bp deletion of the *grp 1.8* promoter fused to the *uidA* gene. A transcription factor (VSF1 for vs-1 binding factor 1) that interacts specifically with *vs-1 in vitro* has been cloned. VSF1 shows homology to the transcription factors of the bZIP family. To demonstrate an interaction of VSF1 and *vs-1 in vivo*, protoplast transfection experiments have been performed. As the reporter construct, we used the *uidA* gene under the control of the -76 bp *grp1.8* promoter downstream of a *vs-1* trimer.Cotrans-fection of the reporter construct with a vector overexpressing VSF1 lead to an 2-3 fold increase in GUS activity. This increase was not detectable when the same promoter without the *vs-1* trimer was used.

S04-39

DIFFERENTIAL EXPRESSION OF SOYBEAN NUCLEAR GENES ENCODING THE CHLOROPLAST SPECIFIC TRANSLATION ELONGATION FACTOR EF-TU

Maurer,F. and Stutz,E., Laboratoire de Biochimie végétale, Université de Neuchâtel, 2000 Neuchâtel

We have shown (Chimia 47: 247-249,1993) that the soybean (*Glycine max.*) nuclear genome contains four genes (*tuf*A1,-A2,-B1,-B2) encoding the chloroplast translation elongation factor EF-Tu. We monitored the expression of all four genes in root, stem and leave tissues using the corresponding total RNA extracts as template in a RT-PCR approach (NAR 20:5861-6862). We find that the genes B1 and B2 are about equally expressed at a relatively low level in all three tissues (constitutive expression) while the mRNAs of genes A1 and A2 are significantly more abundant in aerial tisues than in roots, suggesting that transcription activity and/or transcript stability of A but not of B genes increases in light (modular expression). Indeed A type mRNA concentration sharply increases upon illumination of dark grown seedlings as previuosly shown. We try to define this differential gene expression by comparing the structure and function of A1 and B1 promoter regions. Tobacco plants are transformed with constructs carrying promoters of variable length (deletions) and GUS acitvity is measured. We define the minimal region upstream of the transription start site for promoter activity. The upstream region of A1 (640 pos.) contains regions which may qualify for enhancer-like elements while the B1 upstream region (1141 pos.) lacks equivalent boxes.

S04-40

8.5 Å PROJECTION MAP OF THE LIGHT-HARVESTING COMPLEX I FROM *RHODOSPIRILLUM RUBRUM*

Simone Karrasch[1,2], Per A. Bullough[2], Robin Ghosh[3], [1]M.E. Müller Institute for Microscopic Structural Biology, Biozentrum, Klingelbergstr. 70, CH-4056 Basel, Switzerland, [2]MRC Laboratory of Molecular Biology, Hills Rd., Cambridge CB2 2QH, UK, and [3]Laboratory of Bioenergetics, University of Geneva, Ch. des Embrouchis, 10, CH-1254 Jussy-Lullier/GE, Switzerland

Two-dimensional crystals from the light-harvesting complex I (LHCI) of the purple non-sulphur bacterium *Rhodospirillum rubrum* have been reconstituted from detergent-solubilzed protein complexes. Frozen-hydrated samples have been analyzed by electron microscopy. The crystals diffract beyond 8 Å and a projection map was calculated to 8.5 Å. The projection map shows 16 subunits in a 116 Å diameter ring with a 68 Å hole in the center. These dimensions are sufficient to incorporate a reaction centre *in vivo*. Within each subunit, density for the α and ß polypeptide chains is clearly resolved and the density for the bacteriochlorophylls can be assigned. The experimentally determined structure contradicts models of the LHC I presented so far.

S04-41

THE FAST FLUORESCENCE TRANSIENT OF *RHODOSPIRILLUM RUBRUM* IS LINKED TO THE REDOX PHYSIOLOGY OF THE CELLS.

Reto J. Strasser and Robin Ghosh, Laboratoire de Bioénergétique, Université de Genève, Ch. des Embrouchis, 10, 1254 Jussy-Lullier.

The facultative, non-sulphur purple bacterium *Rhodospirillum rubrum* expresses large amounts of specialized photosynthetic membranes under anaerobic photoheterotrophic or semi-aerobic, chemoheterotrophic conditions. These membranes contain all components of the photosynthetic apparatus necessary for cyclic electron transport. In this study we have examined the kinetics of variable fluorescence arising from the photosynthetic apparatus for wild-type cells growing on different carbon sources and for mutants containing lesions in components involved in electron transport or in the Calvin cycle which fixes CO_2. We show that the variable fluorescence kinetics arising from the photosynthetic apparatus responds directly to redox events in the cell Thus, this technique can be used for biosensing of photosynthetic bacteria in biotechnological applications.

Chromosome structure and regulation of gene expression

S05-01

Maintaining determined states by epigenetic mechanisms

R. Paro, A. Breiling, B. Brückner, V. Orlando, H. Strutt and D. Zink
ZMBH, University of Heidelberg, INF 282, D-69120 Heidelberg, Germany

In Drosophila the genes of the Polycomb-group (PcG) and trithorax-group (trxG) are part of the cellular memory system, maintaining the differential expression patterns of the genes necessary for defining the determined states of the cells. Both groups seem to be acting at the chromatin level. The repressory PcG induces heterochromatin-like structures on genes that need to be stably and heritably inactivated. The role of the trxG is to counteract these special chromatin domains to render the genes accessible to the activating factors. Using an in vivo cross-linking method we were able to map the distribution of the Polycomb (Pc) protein; the trxG protein brahma and a transcription factor Abd-B on different target genes. The activating and the repressing chromatin components show mutually exclusive patterns. The proteins of the PcG act through DNA elements termed PREs. We show that these elements can induce mosaic expression on reporter genes, similar to hetero-chromatin. Using methods of immunocytology we can demonstrate that the PcG-chromatin can prevent an activating factor like the yeast Gal4 from binding to its UAS target. Interestingly, we find that for the function of the PcG protein complex a RNA moiety is needed. We have biochemically isolated a RNA tightly bound to the Polycomb protein through the conserved chromo domain. We have isolated the encoding sequences for the RNA and will discuss the functional implications of the RNA in the silencing process. The PcG in Drosophila appears to develop into an ideal model system to study epigenetic mechanisms of transcriptional regulation, ranging from the silencing of yeast mating type loci to the mechanism of X-chromosome inactivation in mammals.

S05-02

DYNAMIC PROPERTIES OF CHROMATIN AND IMPLICATIONS FOR DNA-REPAIR

F. Thoma, U. Schieferstein, S. Tanaka, R. Wellinger, Institut für Zellbiologie, ETH-Hönggerberg, CH-8093 Zürich.

Packaging of eukaryotic DNA into nucleosomes, chromatin fibers and higher order structures restricts the accessibility of the DNA for proteins and drugs. Since all DNA-dependent processes use chromatin as a substrate, the molecular mechanisms of transcription, replication, recombination and DNA-repair are likely to cause and to be affected by structural changes at the chromatin level. We have studied the properties of nucleosomes and nucleosome positions in yeast minichromosomes and genes. Nucleosomes may be stable, unstable and their positions on the DNA sequence may be altered in vivo. Since DNA-lesions and folding of DNA lead to DNA-distortions, the formation and repair of DNA-lesions may be modulated by the local nucleosome structure. In vitro reconstitution experiments as well as genomic footprinting techniques are currently exploited to investigate the correlation between nucleosome surface and the formation and repair of UV-induced DNA-lesions.

S05-03

Multicomponent Complexes Involved in Chromatin-mediated Gene Repression

Palladino, F., Cockell, M., Gotta, M. and Gasser, S.M.
ISREC, CH-1066 Epalinges

The silent mating type loci and telomere-proximal regions of yeast chromosomes show a position-dependent repression of gene expression that require the N-termini of histones H3 and H4, Silent Information Regulators 2-4, and the telomere-repeat binding protein, RAP1. We have examined the interactions among these proteins by various genetic and biochemical techniques. Telomeres and their associated repressed chromatin appear to be juxtaposed to the nuclear envelope, and disruption of SIR protein-RAP1 interaction or SIR protein-histone interaction, interferes with this subnuclear localization. In particular, overexpression of a coiled-coil domain in the SIR4 C-terminus disrupts telomeric localization and SIR-mediated silencing. We have screened for novel elements mediating these interactions by two-hybrid techniques and by suppression of SIR4 overexpression.

S05-04

CHROMOSOME ANALYSIS OF MONO AND TRIPRONUCLEAR HUMAN OOCYTES PROCEEDED AFTER INTRACYTOPLASMATIC SPERM INJECTION PROCEDURE

E. Macas, B. Imthurn, M. Münch, M. Rosselli, P.J. Keller.
Department of Gynaecology and Obstetrics, Division of Endocrinology, University Hospital Zurich, Switzerland

Recently the techniques of intracytoplasmatic sperm injection (ICSI), using micromanipulation procedure, has been applied in humans for IVF to alleviate severe forms of male infertility. During the micromanipulation procedure the oocytes are in the metaphase stage of the second meiotic division, with the chromosomes located at the periphery of egg. It is still unknown whether the technique of micromanipulation could damage the meiotic spindle, thereby causing the loss of a chromosome(s). The investigation of first cleavage division offer the first opportunity to detect this error. It is well known that after ICSI procedure the certain number of oocytes possess one (parthenogenetic eggs) or three (digynic eggs) pronuclei; these eggs are not destined for replacement and could serve as an alternative model to study the chromosome makeup at the first cleavage division. In our ICSI programme we observed 7.8% oocytes with one and 9.2% with three pronuclei. Seventy one percent of oocytes (17/24) with single pronuclei were successfully karyotyped: 14 were normal, 23,X; two cases were hypohaploid, 21,X,-2G and 20,X,-A,-2C; and one was hyperhaploid, 24,X,+C. Out of 15 oocytes contained three pronuclei, 8 (53%) were successfully analyzed. Five of them had three separate chromosome sets with following complements: 23,X 23,X 23,X; 23,X 23,X 23,Y; 23,X 23,X 23,X; 23,X 26,X,+A,+B,+C 20,X,-A,-B,-C and 23,X 22,X,-C, 24,X,+C. Two chromosome complements of the remaining 3 zygotes were: 23,X 23,X; 24,X,+G 22,X,-G; and 23,X 13. The present data suggest that ICSI procedure may contribute to the irregular segregation of chromosomes at the end of second meiotic division. This suggestive conclusion need to be confirmed by a larger number of specimens.

S05-05

PHYSICAL DETECTION OF HETERODUPLEX DNA MOLECULES FORMED DURING MEIOTIC RECOMBINATION IN THE FISSION YEAST *SCHIZOSACCHAROMYCES POMBE*

Baur M. and J. Kohli, Institute of General Microbiology, Baltzerstrasse 4, CH-3012 Bern

Hybrid DNA with mismatched base pairs is a central intermediate in homologous recombination. In this study, we applied denaturant gel electrophoresis to physically detect heteroduplex DNA formed during meiotic recombination in fission yeast. We analyzed heteroduplex formation at *ura4-A6*, a G to C transversion in the recombination hotspot *ura4-aim* (aim: artificially introduced marker between *ade6* and centromere III). The high level of postmeiotic segregation of this mutant enables mismatch detection. Preliminary results indicate that both possible mismatches (C·C and G·G) are formed during meiosis. While G·G is repaired and thus not detected in late stages of meiosis, C·C escapes correction and is therefore also present at the end of meiosis. The exact timing of heteroduplex formation and mismatch repair in meiosis of *S. pombe* are currently being investigated in more detail. The involvement of Mlh1, a homolog of the bacterial MutL mismatch repair protein, in mismatch correction will also be tested.

S05-06

MSH2 - A NEW *MUTS* HOMOLOGUE OF FISSION YEAST

Rudolph C.[1], Fleck O.[1], Farthmann B.[2], Kramer W.[2], Kohli J.[1]
[1]Institute of General Microbiology, Baltzer-Str.4, 3012 Bern, Switzerland
[2]Institute of Molecular Genetics, Griesebachstr.8, 37077 Göttingen, Germany

DNA mismatches are efficiently repaired by the *mutS* dependent pathway which is highly conserved from bacteria to man. To further investigate the mismatch repair pathways in fission yeast we have cloned a *mutS* homologue. The derived amino acid sequence shows highest homologies to *S. cerevisiae* and human *msh2*. We now construct a gene disruption mutant to analyze the function of *S. pombe msh2* and its involvement in mismatch repair pathways.

S05-07

MEIOSIS IN TOPOISOMERASE II DEFICIENT FISSION YEAST

E. Hartsuiker, J. Bähler and J. Kohli.
Institute of General Microbiology, Baltzer-Str. 4, 3012 Bern, Switzerland.

Complementary temperature shift experiments with a ts *top2* mutant show that topoII is required for both meiotic divisions, early meiotic events are not impaired. At restrictive temperature no altered morphology is visible during meiotic prophase, as judged from electron microscopy and DAPI staining. However, the cells arrest before the first meiotic division. This arrest is not regulatory: in immunofluorescence preparations of arrested cells four separated spindle pole bodies and two spindles (probably representing both spindles of meiosis II) are visible in one nucleus. This striking morphology was never observed at permissive temperature. In a *rec7 top2* double mutant, in which recombination is reduced, the block in meiosis I is partly solved. These results suggest that an important function of topoII during meiosis I is the separation of recombined chromosomes and that topoII does not function in the resolution of interlocks during early prophase.

S05-08

IDENTIFICATION AND CHARACTERIZATION OF DNA TARGET SITES OF THE HUMAN POU PROTEIN mPOU

Wey, E. and Schäfer, B.W., Department of Pediatrics, University of Zürich, Steinwiesstr. 75, CH-8032 Zürich

POU proteins constitute a large family of homeodomain containing proteins. They are characterized by a highly conserved bipartite DNA binding domain which consists of a POU-specific (POU_S) domain and a POU homeodomain (POU_{HD}).
Because little is known about the DNA binding specificities of POU proteins in general and human mPOU in particular, we performed a binding site selection experiment using the fusion protein GST-mPOU and a pool of random oligonucleotides. This approach revealed that mPOU binds preferentially to sequences containing a TAAT motif which is typical for homeodomain containing proteins in general. Furthermore, alignment of selected sequences revealed two consensus motifs: A palindromic motif reading ATTA...TAAT and a motif containing the direct repeat TAAT...TAAT. In order to elucidate whether both subdomains of the POU domain participate in DNA binding, bacterially expressed fusion proteins with either POU_S or POU_{HD} are currently tested in bandshift assays for separate binding of the selected target sites. Furthermore, luciferase reporter constructs containing the consensus motifs are tested as target sites for transcriptional regulation by mPOU in transient transfection assays.

S05-09

Saccharomyces cerevisiae Rad54, a protein involved in recombinational repair and homologous recombination

Beate Clever, Jacqueline Schmuckli, and Wolf-Dietrich Heyer. Institute of General Microbiology, University of Bern, Baltzer-Strasse 4, 3012 Bern.

Several pathways cooperate in the yeast *S. cerevisiae* to repair radiation induced DNA damage. Among these the *Rad52* group genes (*RAD50-57, XRS2, MRE11*) comprise the functions involved in recombinational repair. A representative member of this group is the *RAD54* gene which leads to extreme sensitivity to ionizing radiation when mutated (Game & Mortimer [1974] Mutat. Res. 24, 281-292). Using isogenic strains and *rad54Δ* mutations we now demonstrate that Rad54 is required for mating type switching, a double-strand break mediated recombination event. Furthermore, *rad54Δ* cells exhibit a mutator effect and a reduced rate for spontaneous intragenic recombination in mitosis. The predicted Rad54 amino acid sequence (Emery et al. [1991] Gene 104, 103-106) suggested the existence of two potential nuclear localization signals, a potential leucine zipper, and a non-convential ATP binding site. Mutagenesis of this putative ATP binding site abrogates protein function in DNA repair and mating type switch demonstrating its functional significance. Sequence homologies of Rad54 and a number of other proteins (e.g. *S. cerevisiae* Snf2, Rad5, Rad16, and human ERCC6) in the seven motifs of known helicases suggested the existence of a new, Snf2-subfamily of putative DNA helicases (Gorbalenya et al. [1989] Nucl. Acids Res. 17, 4713-4730). We are currently purifying Rad54 protein to elucidate its biochemical properties.

S05-10

Functional Analysis of a Diversity Element in the Murine Immunoglobulin Heavy Chain Locus

Tiziano Tallone, Sandro Rusconi and Georges Köhler. Max-Planck Institut fü Immunbiologie, Stübeweg 51, D-79108 Freiburg ; * Institut de Biochimie, Rue du Musée 5, CH-1700 Fribourg*

Rearrangement of the IgH gene locus always starts with a D to J joining, followed by the joining of a V gene element. Previous studies suggested that rearrangement regulation is affected by *cis*-acting DNA elements that target gene segments to the recombinase activity. Among the candidate regulatory elements one finds those involved in transcriptional control of the IgH locus. Indeed, in mice rendered deficient for the IgH intron enhancer, the efficiency of the initial D to J rearrangement is only marginally affected, while the efficiency of the V to DJ joining is strongly reduced. These data predict the existence of additional *cis*-regulatory element(s), different from intron enhancer, which are particularly important for D to J rearrangement . A candidate for such a regulatory role has been characterised by Kottmann *et al.* 1994 (*Eur. J. Immunol.* 24, 817-18, and references therein). This *cis*-regulatory element has been mapped within the DQ52 locus and it has been shown to be a complex genetic element interacting with several DNA binding proteins in a tissue-restricted manner. We are investigating the DQ52 element and preliminary data suggest that this element interacts with transcription factors known to be important for several B cell-specific promoters and enhancers functions.

S05-11

Characterization of the mouse homolog to *S cerevisiae* Sep1

Vladimir Bashkirov and Wolf-Dietrich Heyer. Institute of General Microbiology, University of Bern, Baltzer-Strasse 4, 3012 Bern.

S.cerevisiae Sep1 is a multi-functional homologous pairing protein with exonuclease activity and we were interested to clone its mammalian homolog. Protein sequence comparison of *S cerevisiae* Sep1 and its homolog from *S.pombe*, p140^{exo2}, revealed several conserved stretches of amino acids in their NH_2-terminal regions. Using fully degenerated primers and mouse testis cDNA as a template for the PCR, a product with the expected length has been amplified. Cloned mouse PCR-product was used as a hybridization probe for screening libraries and a full length cDNA was assembled. The putative translation product revealed excellent homology to its *S.pombe* and *S.cerevisiae* counterparts. The *mSEP1* sequence was shown to be unique in the genome and the gene is likely to contain many introns. Transcript analysis revealed tissue-specific expression. Western blotting analysis of protein isolated from mouse tissues confirmed the presence of a protein the size predicted by DNA sequence analysis. *mSEP1* was able to complement mutant phenotypes of the *sep1Δ S.cerevisiae* strain. Thus, *mSEP1* is the structural and functional homolog of *S.cerevisiae SEP1*.

S05-12

PRODUCTION OF ACTIVE MOUSE DNA POLYMERASE δ IN BACTERIA

R. Hindges and U. Hübscher. Institute of Veterinary Biochemistry, University of Zürich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland

The entire cDNA for the large subunit of mouse DNA polymerase δ (mPol δ; EC 2.7.7.7) has been cloned and expressed in various bacterial expression systems. A soluble protein could only be obtained when mouse DNA polymerase δ was produced as a glutathione S-transferase fusion protein and the incubation temperature of the expression strains was reduced to 30°C. After purification over a glutathione sepharose column, the fractions containing the recombinant fusion protein showed both DNA polymerase and 3'->5' exonuclease activities. *In situ* activity gel analysis indicated that the polymerase activity reside in the recombinant fusion protein. This activity, however, was not stimulated by proliferating cell nuclear antigen. Our data are discussed in the view of the findings of Goulian et al. (J.Biol.Chem., 265: 16402-16411, 1990) that the second DNA polymerase δ subunit of mouse, the 48 kDa protein, might play an important role in DNA polymerase δ / proliferating cell nuclear antigen interaction.

S05-13

DNA polymerase ε interacts with proliferating cell nuclear antigen in primer recognition and elongation

Giovanni Maga and Ulrich Hübscher
Institute of Veterinary Biochemistry, University of Zürich-Irchel
Winterthurerstrasse 190, Zürich CH

Kinetic analysis of DNA polymerase ε (pol ε) in its interaction with the homopolymeric template-primer poly(dA)/oligo(dT) and a singly-primed synthetic oligonucleotide of defined sequence indicated that primer utilization is inhibited by single-stranded DNA. The K_m values of pol ε for the primer with poly(dA)/oligo(dT) at different template:primer ratios from 5:1 to 30:1 (in nucleotides) ranged respectively from a value of 28 nM to a value of 100 nM. With poly(dA)/oligo(dT) 5:1 in the presence of free ss poly(dA)$_{400}$ the K_m values were ranging from 30 nM for a ssDNA:primer ratio of 5:1 to 110 nM for a ratio 30:1. Different ss DNA molecules of increasing length in single-standness inhibited pol ε in a competitive mode with the 3'-OH primer. The calculated K_i values for the ss DNA decreased as the single-strandness length increased. Thus, long single-stranded DNA regions appear to sequester pol ε via non-productive binding, reducing its catalytic efficiency. Proliferating cell nuclear antigen (PCNA) can reduce this non-productive effect. In the presence of PCNA, the K_m value of pol ε for the primer with poly(dA)/oligo(dT) at a molar ratio of 30:1 was reduced from 100 nM to 13 nM. Direct measurements of the formation of the complex between pol ε and a singly primed oligonucleotide by a gel retardation assay, showed that PCNA increased the rate of primer binding (k_{on}) of pol ε without affecting the rate of dissociation of the pol ε:primer complex (k_{off}). Once the complex between pol ε and the primer is formed, PCNA can increase the rate of processive nucleotide incorporation (k_{cat}). The results suggested a dual role of PCNA in stimulating the activity of pol ε, namely, first to facilitate primer binding and second to stimulate the synthetic activity.

S05-14

β-Actin as an intracellular target for the large subunit of HIV-1 and FIV reverse transcriptase

Michael Hottiger, Mario Amacker, Kosi Gramatikoff[1], Oleg Georgiev[1], Walter Schaffner[1] and Ulrich Hübscher
Department of Veterinary Biochemistry and [1]Molecular Biology II, University Zürich-Irchel, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland

A filamentous phage cDNA expression library from human lymphocytes was used to select cellular proteins interacting with HIV-1 reverse transcriptase. Affinity selections by using the bacterially expressed large subunit of reverse transcriptase from HIV-1 yielded host β-actin. The interaction was characterized by using [^{32}P]-labeled reverse transcriptase and purified GST-β-actin. Finally, we show that also the large subunit of FIV reverse transcriptase interacts with GST-β-actin and that β-actin is part of highly purified retroviruses. This finding is intriguing in the view of recent observations that HIV-1 virions are secreted unidirectionally on the tip of a cell pseudopod which itself is formed by actin. Therefore reverse transcriptase/β-actin interaction might be important for focusing Gag-Pol precursors into actin based pseudopods involved in cell-to-cell transmission of lentiviruses such as HIV-1 and FIV.

S05-15

Functionally expressed subunits of FIV reverse transcriptase possess strand displacement activity

Mario Amacker, Michael Hottiger and Ulrich Hübscher
Department of Veterinary Biochemistry, University of Zurich, Winterthurerstr. 190, CH-8057 Zurich, Switzerland

The 51 and 66 kDa subunits of reverse transcriptase (RT) from FIV have been cloned and functionally expressed in *Escherichia coli* as glutathione-S-transferase (GST) - fusion proteins. The homodimers enzymatically cleaved from the GST part were functionally characterized and compared to HIV-1 RT. In analogy to the recently described strand displacement activity of HIV-1 RT (Hottiger, M., Podust, V. N., Thimmig, R. L., McHenry, C. and Hübscher, U.; J. Biol. Chem. 269, 966-991, 1994) we show that the FIV RT also performs strand displacement DNA synthesis. For this purpose we have constructed a novel DNA substrate with a defined nick. We propose that this activity might be a promising traget to screen for a new generation of RT inhibitors.

S05-16

ASSEMBLY OF DNA POLYMERASE δ AND ε HOLOENZYMES DEPENDS ON THE GEOMETRY OF THE DNA TEMPLATE.

Podust L.M., Podust V.N., Floth C., Hübscher U.
Department of Veterinary Biochemistry, University Zürich-Irchel, Switzerland.

To study in details the assembly of DNA polymerases δ and ε holoenzymes a circular double-stranded DNA template containing a gap of 45 nucleotides was constructed. Both replication factor C and proliferating cell nuclear antigen were absolutely required and sufficient for assembly of DNA polymerase δ holoenzyme complex on DNA. On such a circular DNA substrate replication protein A (or *E.coli* single-strand DNA binding protein) was neither required for assembly of DNA polymerase δ holoenzyme complex nor for the gap-filling reaction. A circular structure of the DNA substrate was found to be absolutely critical for the ability of auxiliary proteins to interact with DNA polymerases. The linearization of the circular DNA template resulted in three dramatic effects: (i) DNA synthesis by DNA polymerase δ holoenzyme was abolished, (ii) the inhibition effect of replication factor C and proliferating cell nuclear antigen on DNA polymerase α was relieved and (iii) DNA polymerase ε could not form anylonger a holoenzyme with replication factor C and proliferating cell nuclear antigen. The comparison of the effect of replication factor C and proliferating cell nuclear antigen on DNA polymerases α, δ and ε indicated that the auxiliary proteins appear to form a mobile clamp, which can easily slide along double-stranded DNA.

S05-17

POLY-GLUTAMINE STRETCHES AND TRANSCRIPTION.

Patrick Rigoni and Sandro Rusconi, Institut de biochimie, Université de Fribourg, rue du musée 5, 1700 Fribourg

CAG repeats are often found in the coding sequences of transcription factors, where they are mostly translated as poly-glutamines. It has been shown that the length of these monotonous stretches can affect the transactivating activity of the factors both in a positive and in a negative manner depending on the specific context. In addition, in the last few years four neurodegenerative disorders have been linked to the presence of expanded forms of these repeats. We assume that poly-glutamine may be the target of some cellular factor which act negatively on certain promoters. We want to screen a cDNA library with a two-hybrid system for the presence of poly-glutamine interaction using poly-glutamine targets of two different lengths. Positive interacting clones will then be tested on transcriptional assays for their specificity, biological and medical significance.

S05-18

FORMALDEHYDE FIXATION OF NUCLEI AS A METHOD TO INVESTIGATE CHROMATIN STRUCTURE AND METHYLATION OF RIBOSOMAL RNA GENES

I.Stancheva, R. Lucchini, Th. Koller, J.M.Sogo
Institute fur Zellbiologie, ETH-Hönggerberg, 8093 Zurich

Formaldehyde fixation is known to crosslink in chromatin histones to DNA(Solomon et al.,1985,PNAS 82). We used this technique on rat liver nuclei in order to distinguish between the two populations of actively transcribed and inactive rRNA gene copies, which might have different amount of crosslinkable histones. Formaldehyde mediated crosslinking in transcribed and silent genes is detected in a gel retardation assay. This technique has the advantage that achieved protein-DNA complexes can be easily decrosslinked and used for further studies. Formaldehyde fixation was tested using a well established method - psoralen photocrosslinking (Conconi et al.,1989,Cell 57). Next we applied both formaldehyde and psoralen crosslinking techniques to study the methylation of rRNA genes in rat liver nuclei and several rat cell lines taking in account the advantage of each technique. This approach allowed us to investigate separately active and inactive of rRNA coding regions and their upstream enhancer sequences.

S05-19

V(D)J RECOMBINATION:EFFECT OF CIS-ACTING ELEMENTS

Roch,F.A.,Hobi,R.,Berchtold,M.W.,& Kuenzle,C.C.
Institute of Veterinary Biochemistery, University of Zürich,Winterthurerstrasse 190, CH-8057 Zürich

Rearrangement of immunoglobulin genes occurs in the early stages of B-(or T)cell ontogeny and is turned off once a functional antigen receptor is expressed. It has been proposed that transcriptional enhancers, may act on the regulation of V(D)J recombination. Here we focused on the mouse heavy chain enhancer (Eμ) composed of a "core enhancer" flanked by 2 MARs (Matrix Attachment Regions). Eμ, and subfragments of it, were inserted between the recombination signal sequences (RSS) interrupting the lacZα gene of plasmid pBlueRec. Other putative cis-acting sequences were inserted at the same site: MARs (human, chicken, Drosophila), viral enhancers from CMV or SV40, and control elements lacking promoter/ enhancer or MAR activity. After transient transfection into a pre-B cell line, recombination frequency (Rfq) was measured by the ratio of blue to total number of colonies on X-gal/IPTG/Amp plates. Here we show: 1-Eμ insert, the human MAR MII and the promoter element E3 induce a 3-fold increase of pBlueRec basal Rfq ($p<0.01$). 2-MAR activity *per se* did not correlate with V(D)J up- or down- regulation. 3-One HinfI-XbaI subfragment of Eμ located downstream of the "core enhancer" carries most of the upregulating effect of Eμ.

S05-20

LOW X-RAY DOSE INDUCED LOSS OF HETEROZYGOSITY IN *DROSOPHILA*

P. M. Schweizer
Institute for Medical Radiobiology of the University of Zürich and the Paul Scherrer Institute, August Forel-Str. 7, CH-8029 Zürich, Switzerland

Homologous recombination has emerged lately as a surprisingly common cause of recessive functional gene loss in mammalian cells and has been implicated in tumor suppressor gene loss in human neoplasms. In *Drosophila*, X-ray-induced loss of heterozygosity at the cell marker loci *mwh, flr* and *zw3*tic is enhanced by a factor of 2 over control level following exposure of larvae to doses as low as 0.01, 0.03 Gy or 0.1 Gy of X-rays. The frequencies of recombinant clones induced with eight doses in the interval 0.01 - 2.0 Gy is linearly related to the dose. The regression lines show no significant intercept at zero dose. Conditions of chronic irradiation at dose-rate of 15.7x10E-5 Gy/min provided no evidence of an inverse dose-rate effect as reported for homologous recombination in yeast (Kiefer *et al.*, 1988). In human B-lymphoblastoid cells Benjamin and Little (1992) reported approximately 5x10E-8 recombination/Gy inter-allelic recombination in the thymidine kinase locus between exon 4 and exon 7 separated by 8 kb. So one recombination per Gy was induced per 8kbx5x10E+8 = 4x10E+9 kb irradiated DNA. Compared to one recombination per Gy per 1.02x10E+8 kb calculated from recombinations proximal to *mwh* and one recombination per Gy per 0.88x10E+8 kb calculated from recombinations proximal to *flr* in *Drosophila* a ratio of 40:1 recombination/Gy/kb in *Drosophila* versus human results. In *Drosophila melanogaster*, the probability of homologous recombination per induced DNA double-strand break appears to be at least one order of magnitude higher than in man. Ref.:

KIEFER J. *et al.*, 1988, *Radiation Research*, 113,71-78. BENJAMIN, M. B. AND LITTLE, J.B. 1992, *Molecular and Cellular Biology*, 12, 2730-2738.

S05-21

NUCLEOSOME STRUCTURE AFFECTS FORMATION OF PYRIMIDINE DIMERS IN OLIGO(DT) TRACTS

Uwe Schieferstein and Fritz Thoma, Institut für Zellbiologie, ETH-Hönggerberg, CH-8093 Zürich

To study the relationship between nucleosome structure and formation of UV-induced DNA lesions, mononucleosomes were reconstituted on a DNA sequence containing several oligo(dT) tracts. The DNA was irradiated with UV light (254nm) either before or after reconstitution. Nucleosomes were characterized by DNaseI digestion, formation of cyclobutane pyrimidine dimers (CPDs) was detected by a pyrimidine dimer-specific endonuclease (T4-endoV).
While reconstitution of undamaged DNA produced rotationally positioned nucleosomes, positioning was lost when UV-irradiated DNA was used for reconstitution. In contrast, irradiation after reconstitution did not change positioning but resulted in an altered CPD pattern compared to that of irradiated naked DNA. The modulated CPD pattern was different from those reported for bulk nucleosomes indicating distinct properties of the oligo(dT) structure on the nucleosome.

S05-22

β–Galactosidase α-Complementation in Eucaryotic Cells

P. Moosmann[1] and S. Rusconi[2]
[1] Institut für Molekularbiologie II der Universität, Winterthurerstrasse 190, 8057 Zürich
[2] Institut de Biochimie, Rue du Musée 5, 1700 Fribourg

α–Complementation, i.e. the ability of a N-terminal fragment of ß-galactosidase to form an active complex with an inactive deletion mutant of the same enzyme, has been used for several decades to identify recombinant DNA in procaryotes (1). This property is due to the special function of the first 50 amino acids in formation of the activating interface (2). In order to have a simple test for expression of a sequence of interest, e.g. as a fusion protein, we applied this principle to eucaryotic cells. We found that the α–complemented ß-galactosidase is equally active, if not more, than the full length enzyme. Analogously to the bacterial system, the use of α-complementation in appropriate cell lines (or even transgenic animals) may allow direct monitoring of gene expression levels, while circumventing the problem of long fusion proteins or long bicistronic mRNAs.

1. Ullmann, A., Jacob, F. and Monod, J. *J molec. Biol.* **24**, 339-343 (1967).
2. Jacobson, R.H., Zhang, X-J., DuBose, R.F. and Matthews, B.W. *Nature* **369**, 761-766 (1994).

S05-23

IMAGING THE FRAGILE X WITH THE SCANNING FORCE MICROSCOPE (SFM)

Hämmerle S.[1], Froster U. G.[2], Niederer P.[1], Anliker M.[1], Walt H.[2]

[1] Institute of Biomedical Engineering and Medical Informatics, University and ETH, Zürich; [2] Department of Obstetrics and Gynaecology University Hospital, Zürich

Fragile X syndrome is a well established genetic disorder which is accompanied by mental retardation, macroorchidism and behavioural anomalies. The reason is a mutation located on the X-chromosome consisting of an elongation of a $(CGG)_n$ trinucleotid repeat. We succeeded in imaging the associated fragile site at Xq27.3 with SFM using slides prepared for conventional cytogenetical analysis. This is the first study of chromosomal anomaly with SFM. The image quality opens ways for more detailed information in chromosome analysis.

S05-24

RAPID CHANGES IN CHROMATIN CONFIGURATION AFTER DNA DAMAGE

Saydan, N., Kohler, M., Heimgartner, E., Jaussi, R., Institut für Medizinische Radiobiologie, Paul Scherrer Institut & Universität ZH, CH-5232 Villigen-PSI

The effect of X-ray-induced DNA damage on chromatin configuration was assessed with Fluorescence In Situ Hybridisation (FISH). The influence of the damage on the area of signals from the centromeres of a TK6 human lymphoblast cell line was quantified. Cells were irradiated with 2 Gy X-rays and fixed at 0h and 12h after irradiation. Further cell samples were treated with caffeine after irradiation. The cell cycle status in each of the samples was analysed by flow cytometry. An alpha-satellite DNA hybridisation probe was used for FISH. Digital fluorescence images were obtained with a confocal laser scanning microscope and analysed by a specific software. Our preliminary results indicate that the average centromere spot size is larger immediately after irradiation compared to that of control cells. The enlargement persists for at least 12h. On caffeine treatment, the enlargement is abolished. Flow cytometric analysis showed the same DNA histograms for cells that had been irradiated and immediately fixed as for unirradiated controls. We conclude that the enlarged spot sizes are not due to a radiation-induced accumulation of cells having the double amount of DNA (late G2), but that they rather represent a rapid radiation-induced effect.

S05-25

Immediate-early- (IEG) and heat-shock-protein- (HSP) gene expression after a single bout of exercise in humans

A. Puntschart, M. Vogt, , H. Heider, H.-R. Widmer, K. Jostarndt, H. Hoppeler, R. Billeter
University of Bern, Department of Anatomy, Bühlstr. 26, 3012 Bern, Switzerland

The response of eukaryotic cells to different challenges is generally associated with the expression of a set of genes which are referred to as immediate early genes (IEGs). Stress proteins, also called heat shock proteins (HSPs), comprise a family of proteins that are rapidly induced upon a variety of stimuli, e.g. heat or oxidative stress. We hypothesize that a single exercise bout is sufficient to induce changes in the regulatory network of muscle cells which ultimately lead to changes in the steady state levels of mRNAs responsible for structural and functional adaptations to exercise which have been described before.

In a preliminary study, we have followed the expression of c-fos and HSP70 in the hours after a single exercise bout, using a PCR approach for the quantitation of these mRNAs. An untrained subject ran on the treadmill during half an hour at a speed equivalent to his anaerobic threshold. Biopsies were taken before as well as 7min, 30min, 3hrs, 8hrs and 48hrs after the run. The contents of c-fos and HSP70 mRNAs were upregulated 3- and 4fold, respectively, at 7min. 30min after cessation of the run, c-fos and HSP70 mRNA were 6- and 8fold higher than before the exercise. Three hours after the exercise, c-fos expression had returned to the control level, whereas the induction of HSP70 mRNA declined somewhat slower.

We are currently extending this preliminary study, including the quantitative determination of the corresponding protein concentrations. In addition, the measurement of a set of selected kinases, which might be activated in response to exercise, will be included.

S05-26

REPLICATION OF TRANSCRIPTIONALLY ACTIVE CHROMATIN

Renzo Lucchini and José M. Sogo, Institute of Cell Biology, ETH-Hönggerberg, CH 8093 Zürich.

In the yeast *Saccharomyces cerevisiae*, replication machinery entering a transcriptionally active, nonnucleosomal rRNA transcription unit generates two newly replicated coding regions regularly packaged in nucleosomes indicating that the active chromatin structure cannot be directly inherited at the replication fork. The open chromatin conformation is reestablished post-replicatively, most probably through the action of newly initiated RNA polymerase molecules disrupting nucleosomes in their path. The rapidity with which some newly replicated rRNA gene promoters are assembled into a nonnucleosomal structure following replication does not exclude the possibility of an assembly of potentially active promoters at the replication fork. However, for a considerable fraction of the newly replicated rRNA genes the establishment of an exposed chromatin conformation at their promoters does also occur later by a mechanism involving disruption of preformed nucleosomes.

S05-27

STRUCTURAL ORGANIZATION OF TRANSCRIPTIONALLY ACTIVE rRNA GENES AND THEIR REGULATORY SEQUENCES IN YEAST

Reinhard Dammann, Renzo Lucchini, Theo Koller and José M. Sogo, Institute of Cell Biology, ETH-Hönggerberg, 8093 Zürich

In *S. cerevisiae* the transcriptionally active rRNA genes are organized in clusters comprising two to three gene copies. A detailed analysis of the enhancer element revealed a strict correlation between the chromatin structure of the enhancer and the transcriptional activity of the flanking upstream gene: nucleosome-free enhancers are always preceded by active rRNA gene copies, whereas nucleosome-packed enhancers by inactive genes. In contrast, this correlation is not found for the gene localized downstream of the enhancer, suggesting that enhancers act mainly on the most proximal upstream promoters. Nucleosome-free enhancers are also present in a RNA polymerase I mutant strain and seem to be independent of transcription. In this mutant, the rRNA coding sequences are packaged in nucleosomes. These data suggest that preactivation of rRNA genes is not sufficient for the disruption of nucleosomes on the coding region. The establishment of the active ribosomal chromatin devoid of nucleosomes requires RNA polymerase I advancing through the template.

S05-28

ENTRY-EXIT OF DNA IN THE H_1-FREE NUCLEOSOME DETERMINED BY CRYO-ELECTRON MICROSCOPY

Furrer P., Bednar J., Hamiche A.*, Prunell A.* and Dubochet J.

Laboratoire d'Analyse Ultrastructurale, Université de Lausanne, CH-1015 Dorigny
*Institut Jacques Monod, Université Paris 7, 2 Place Jussieu, Tour 43, F-75251 Paris-Cedex 05

Whereas the DNA path inside the nucleosome is well established, it is essentially unknown in the "entry-exit" region. The 3-dimensional (3D) structure of "linker" DNA was investigated here on single nucleosomes reconstituted without H1 on a 256 base-pair (bp) DNA fragment. Cryo-electron microscopy (cryo-EM) and 3D-reconstruction reveal these nucleosomes as 1.66 left-handed superhelical turn particles. However, the DNA does not enter or leave the particle tangentially, but bends away from it, thus preventing the occurrence of a crossing in the entry-exit region.

S05-29

ARTEFACTS OF CLASSICAL PREPARATION METHODS FOR TRANSMISSION ELECTON MICROSCOPY FOLLOWED STEP BY STEP USING CRYOELECTRON MICROSCOPY.

Salamin Michel L. and Sartori N. LAU,Uni Lausanne

Cryosections of human lymphocytes either in their native frozen hydrated state or after chemical fixation are obtained using high pressure freezing followed by cryoultramicrotomy and directly observed in a cryo-electron microscope. In its native state,the cell shows the usual weakly contrasted aspect typicall of vitreous sections. Nuclear chromatin is finely granulated,the nuclear and cytoplasmic membranes are well delineated and well contrasted. (2%) Glutaraldehyde fixation does not induce any visible damage whereas posttreatment with (1%) OsO_4 brings dramatic changes. The chromatin fine granulation turns up to fibrilar structures randomly oriented. The nuclear membrane is hardly visible and the cytoplasmic one, brittle. Further dehydration with (50%) ethanol leads to a complet disapearance of image contrast, bubbling under the electron beam being then necessary to visualize gosts of the membranes. At the end of the process, cells are embedded in Epon and the classical view is obtained!

S05-30

ELECTRON MICROSCOPIC VISUALIZATION OF RUVA AND RUVB DNA COMPLEXES INVOLVED IN THE PROCESS OF DNA STRAND EXCHANGE.

Stasiak, A., Laboratoire d'Analyse Ultrastructurale, Université de Lausanne, CH-1015 Lausanne-Dorigny, **Egelman, E.H.,** Dept. of Cell Biology and Neuroanatomy, University of Minnesota, Minneapolis, MN 55455, USA, **Parsons, C.A, Bennet, R.J. & West, S.C.** Imperial Cancer Research Fund, Clare Hall Laboratories, Herts., EN6 3LD, England.

In all organisms, genetic recombination relies upon formation of heteroduplex DNA. Electron microscopic visualization of two bacterial proteins, RuvA and RuvB, which promote the branch migration of a crossover, or Holliday junction, provides new insight into the enzymatic mechanism of this process. RuvA oligomer binds the crossover and directs the assembly of two hexameric RuvB rings on the Holliday junction which assumes a square-planar configuration. We propose a molecular model for branch migration, a novel feature of which is the role attributed to two hexameric rings of RuvB which are tethered to each other through their interaction with RuvA. In this model, the two RuvB rings which are diametrically opposed across the Holliday junction, each encompass a DNA duplex and drive branch migration by catalysing the helical rotation of DNA.

S05-31

FUNCTION OF THE *DROSOPHILA* E78 B TRUNCATED STEROID RECEPTOR

*G. Heimbeck *, A.T.C. Carpenter‡, C. Martin ‡, ,S.R.H. Russell ‡ & M. Ashburner‡, * Zoologisches Institut, Universität Freiburg, CH-1700 Freiburg, ‡ Department of Genetics, Cambridge, U.K.*

Gene activity in response to the insect steroid homone, 20-hydroxy-ecdysone, is often associated with the formation of puffs in salivary gland chromosomes. A highly coordinated sequence of more than 100 puffs can be observed *in vivo* and *in vitro* in response to hormone.The *E78* gene, localized within the early-late puff 78C, encodes two different forms of a nuclear hormone receptor, E78A and E78B. E78A contains two zinc fingers and is a member of the group II nuclear hormone receptors. E78B is a truncated receptor protein, lacking the putative DNA binding domain. Only *E78B* transcripts are detected in salivary glands of late third instar larvae and white prepupae at the onset of metamorphosis. Ablation of both, the *E78A* and *E78B* transcripts has no consequences for fertility or viabililty under laboratory conditions. However, mutants deficient for *E78B* transcripts as well as inversions reducing the level of *E78B* transcription show a reduction in the size of a few well characterized late puffs. Ectopic expression of *E78B* under a hs promoter has a similar effect on puffing, showing a domininat negative phenotype. Puffing is not abolished completely, suggesting redundancy in the system. We assume that the truncated E78B receptor heterodimerizes with another, as yet undetermined, steroid receptor and thus exerts its role in puffing and possibly in transcriptional regulation of a subset of genes in salivary glands.

S05-32

A screen for developmentally regulated genes in the zebrafish using DDRT-PCR

Andreas M. Vogel, Caroline Bornmann, Giselbert Hauptmann, Philipp Spaniol and Thomas Gerster, Biozentrum, Abt. Zellbiologie, 4056 Basel

The recently described technique of differential display reverse transcription-PCR (DDRT-PCR) provides a tool to screen for differentially expressed genes. Similar to subtractive hybridization procedures, DDRT-PCR allows the comparison of mRNA populations. However, DDRT-PCR is more versatile, since more than two samples can be compared, and both repressed and induced transcripts can be detected simultaneously.

In an attempt to isolate new genes with a regulatory function during early development of the zebrafish, *Danio rerio*, we are making use of DDRT-PCR. Bands that show differential expression on a DDRT-PCR gel are isolated, amplified and tested for tissue-specific expression by whole mount *in situ* hybridization to zebrafish embryos. In a pilot screen, the conditions for DDRT-PCR using RNA from various stages of the first day of development (i.e. between fertilization and early organogenesis), have been established. The differential expression of band amplificates has been confirmed on Northern blots and by whole mount *in situ* hybridization. In the course of these pilot experiments, a differential band amplificate has been found that shows specific expression in the forming axial and paraxial mesoderm.The corresponding gene is currently being characterized in more detail.

As soon as the conditions for a DDRT-PCR-based screen are fully established, we will undertake a full-scale screen for developmentally regulated zebrafish genes using this technique.

S05-33

A MUTANT HSV-1 SPECIFYING A FUNCTIONAL TK-FUSION PROTEIN

Geng Y. and Ackermann M. Inst Virol, Uni Zurich

We have previously reported a recombinant HSV-1 which expresses HSV-1 ICP0 homologue in BHV-1. The recombinant was constructed which contained the BICP0 gene under its native promoter in the tk locus of HSV-1 and which expressed a truncated form of TK because of the insertion at nearly middle of tk gene. Marker rescue experiment was performed in order to restore wild-type TK with a plasmid containing the entire BamHI-Q fragment of HSV-1. After cotransfection of plasmid and recombinant HSV-1 DNA into Vero cells, viral isolates were selected through three rounds of HAT selection and plaque purification. Genotypic and phenotypic analyses revealed a mutant HSV-1 with the following characteristics: (i) TK^+; (ii) $BICP0^-$; (iii) $syncycium^+$, inheritant from its parent; (iv) larger BamHI-Q fragment named Züri-Q (4.3 instead of 3.6 kb); (v) expressed a TK-fusion protein (Züri-TK) migrating with Mr 65 K instead of 37 K in SDS-PAGE.

S05-34

EXCISION REPAIR OF BULKY DNA ADDUCTS FORMED BY GENOTOXIC ANTIBIOTICS

D. Gunz and H. Naegeli, Institute of Pharmacology and Toxicology, University of Zürich-Tierspital

Excision repair is an important mechanism to remove DNA damage and maintain the genetic integrity. Nucleotide excision repair, in particular, is believed to be absolutely required to process bulky DNA adducts. We have investigated the DNA repair responses to bulky adducts *in vitro* by incubating damaged plasmids with human cell extracts that were prepared using published procedures. Repair synthesis was monitored by the incorporation of radiolabeled nucleotides into damaged DNA. We observed that bulky adducts formed by the natural antibiotics CC-1065 and anthramycin induce damage-specific DNA synthesis in extracts from HeLa and HaCaT cells. Unexpectedly, high levels of DNA repair synthesis were also observed in response to these agents in nucleotide excision repair-deficient extracts from xeroderma pigmentosum cells. This finding suggests the existence of an alternate pathway in human cells to process bulky adducts induced by these and other genotoxic antibiotics. The molecular details of this mechanism are currently under investigation. (Supported by NF 31-40307.94 and the Wolfermann-Nägeli-Stiftung)

S05-35

CONSTRUCTION OF PLASMID DNA CONTAINING SITE-DIRECTED NUCLEOTIDE MODIFICATIONS

B. Zweifel and H. Naegeli, Institute of Pharmacology and Toxicology, University of Zürich-Tierspital

Structural alterations of DNA have been shown to affect essential pathways of nucleic acid metabolism such as replication or transcription. To study the responses of DNA processing enzymes to DNA modifications, we decided to construct plasmids containing altered nucleotides at unique positions. For that purpose, we have exploited a widely used strategy for the preparation of site-specifically modified genomes involving the ligation of modified oligonucleotides into gapped M13 derivatives. Using this procedure, we have prepared plasmids containing a single acetylaminofluorene-guanine adduct placed in the *Bst*BI recognition sequence (5'-TT'CGAA). The presence of bulky adducts in the expected location was confirmed by incubation with *Bst*BI. Cleavage by this restriction enzyme was completely blocked by the site-directed acetylamino-fluorene-guanine adduct. We are planning to use this system to synthesize plasmids containing site-directed DNA-protein crosslinks or various chemical modifications in the deoxyribose residue of DNA. (Supported by NF 31-40307.94 and the Wolfermann-Nägeli-Stiftung)

S05-36

ELECTRON MICROSCOPIC ANALYSIS OF TRANSCRIBING CHROMATIN.

ten Heggeler-Bordier B, Schild-Poulter C and Wahli W. IBA Université de Lausanne.

Electron microscopy was used to monitor the fate of reconstituted nucleosomes during *in vitro transcription* by the T7 RNA polymerase and RNA polymerase II from rat liver nuclear extract.With the purified T7 RNA polymerase the nucleosomes disappeared in the transcribed region of the pSG5 template but in the *in vitro transcription* of a vitellogenin gene from Xenopous Laevis with RNA polymerase II in a rat liver nuclear extract,the nucleosomes persisted on the template. Nucleosomes also disappeared on a 20-fold repeat of the 5S rRNA gene of the sea urchin Lytechinus variegatus cloned downstream of the T7 promoter of the plasmid BS/KS+-. When nuclear extract was added to the *in vitro transcription* reaction with the T7 RNA polymerase and the 20-fold repeat of the 5S rRNA gene, the nucleosomes did also stay on the template. These results suggest that a factor in the nuclear extract is responsible for the maintenance of the nucleosomes during *in vitro transcription*.

S05-37

ANALYSIS OF THE REQUIREMENT FOR MISMATCHED NUCLEOTIDES IN THE 5' TERMINAL HAIRPIN OF MVM DNA

Eithne Costello, Roland Sahli, Bernhard Hirt and Peter Beard.
ISREC, 1066 Epalinges, Switzerland.

The 5' terminal palindrome of MVM can assume a hairpin-like structure, the stem of which is entirely base-paired, except for three unpaired nucleotides which form a bubble. In order to assess the importance of the bubble for viral replication, we generated an MVM mutant lacking a bubble (MVMx) and one with a bubble of altered nucleotide sequence (MVMs). Although MVMx can be propagated, it is defective in comparison with wild-type MVMp. It exhibits a lower ratio of plaque forming units to particles than the wild-type. In mixed infections, MVMx was consistently outgrown by MVMp. Following infection with an equivalent number of particles the rate of accumulation of intermediates of DNA replication was lower for MVMx than for the wild-type virus. Analysis of the 5' termini of replicative form DNA suggested that the ability of MVMx to convert hairpin 5' termini to extended termini is impaired. In contrast, the virus which retained a bubble, though of altered nucleotide sequence, MVMs, behaved like MVMp in all the above assays. We conclude that while a bubble in the 5' terminal hairpin of MVM DNA is not strictly required for viral growth in tissue culture, specific alterations in the nucleotide sequence at the bubble region can result in a virus which is less infectious and has a selective disadvantage compared to the wild-type. The lower infectivity of MVMx may be related either to the inability to make a bubble or to the altered DNA length or sequence when compared to the wild-type DNA. This region apparently contributes to the amplification of viral DNA replicative intermediates.

S05-38

STUDIES ON HUMAN PAPILLOMAVIRUS TYPE 18

Y. Stauffer, F. Hoffmann, L. Pizer, J. Mirkovitch, E. Offord, and P. Beard
ISREC, 1066 Epalinges, Switzerland

I. SYNTHESIS OF VIRAL CAPSID PROTEINS IN A VACCINIA-BASED EXPRESSION SYSTEM. HPV18 is associated with genital carcinoma. Like other human papillomaviruses, HPV18 cannot be easily propagated in cell culture. Moreover, very little viral capsid protein is found *in vivo* in infected tissues. The L1 and L2 genes code for the major and minor capsid proteins, respectively. To produce capsid proteins, we made constructs with the two genes for expression in vaccinia virus. Three recombinant vaccinia viruses expressing L1, L2 and both proteins together were analysed by in vivo labelling, immunoprecipitation and immunoblotting experiments. Both proteins are synthesised and translocated to the nucleus. We expect the L1 and L2 proteins expressed together to self-assemble into virus-like particles.

II. ANALYSIS OF HPV18 TRANSCRIPTION BY NUCLEAR RUN-ON AND FOOTPRINTING ASSAYS With the aim of understanding which factors control the *in vivo* activity of the HPV18 E6/E7 promoter in C4-1 cervical tumour cells, we set out to modulate the activity of this promoter and compare with changes in the proteins bound to it as measured by genomic footprinting. The rate of transcription was measured in a "run-on" assay, and the RNA level by an RNase protection assay. The glucocorticoid hormone dexamethasone stimulated transcription, as did the tumour promoter TPA and the phosphatase inhibitor okadaic acid. Retinoic acid reduced the rate of transcription from the HPV18 promoter.

C4-1 cells were lysed and the isolated nuclei digested with DNase I. The pattern of DNase digestion of the HPV18 DNA in nuclei, when compared with the pattern obtained with naked DNA showed a series of sensitive sites and protected areas associated with the regions containing transcription factor binding sites. Because dexamethasone, okadaic acid and retinoic acid affect rates of HPV mRNA synthesis analysis of their effects on proteins at the promoter *in vivo* should be of interest.

S05-39

EXPRESSION OF THE ALPHA 2-ANTIPLASMIN GENE IN ADULT MURINE TISSUES.

Menoud PA, Khoshbeen M, Sappino N, Vassalli J.-D and Sappino AP. Department of Medicine and Morphology, Geneva Medical School, CH-1211 Genève 4, Switzerland.

The net proteolytic activity catalyzed by proteolytic cascades results from a balance between the production of proteases and antiproteases. The activity of the plasminogen activator (PA)/plasmin system is controlled by inhibitors directed against PAs and by alpha 2-antiplasmin (α2-AP), a plasma serpin with high specificity and reactivity towards plasmin. Contrary to PA inhibitors, little is known about the α2-AP. As a first step to explore the in vivo contribution of α2-AP, we have cloned the murine α2-AP cDNA and analyzed its mRNA distribution in various tissues by in situ hybridization and Northern blot analysis. As expected, α2-AP mRNA is most abundant in liver. Surprisingly, we also found α2-AP mRNA in additional organs such as kidney, intestine and hippocampus. In the kidney, we localized α2-AP mRNA exclusively in cortical tubules. Furthermore, α 2-AP mRNA expression showed a sexual dimorphism and was found to be hormone dependent. Our observations suggest that α2-AP, in addition to being a plasma antiprotease could function as an important distal regulator of plasmin-mediated extracellular proteolysis.

S05-40

NONCOVALENT BINDING OF POLY(ADP-RIBOSE) TO SELECTED NUCLEAR MATRIX PROTEINS

M. Malanga, H.E. Kleczkowska & F.R. Althaus
Institute of Pharmcology & Toxicology - University of Zürich - Tierspital

Poly(ADP-ribose) can modulate chromatin structure through covalent binding and/or non covalent interactions with nuclear proteins. Using a rapid screening technique we have recently identified histones as the predominant poly(ADP-ribose)-binding species in nuclear lysates from different sources. In this study we extended our analyses to nuclear matrix proteins from mammalian cells. Results showed that specific proteins in the nuclear matrices isolated from mouse lymphoma cells (L5178Y) and human keratinocytes (HaCaT) exhibit high affinity for ADP-ribose polymers. These interactions were detected independently of the procedure used for matrix isolation and resisted high salt treatment (up to 2M NaCl). Our observations raise the possibility that poly(ADPR) mediates the association of specific nuclear proteins with nuclear matrix components.

S05-41

POLY(ADP-RIBOSE) METABOLISM IN L5178Y CELLS EXPOSED TO IONIZING RADIATION

H.E. Kleczkowska[1 2], F.R. Althaus[1] and I. Szumiel[2]
[1]Institute of Pharmacology and Toxicology, Univ. Zürich - Tierspital, CH8057 Zürich, and [2]Institute of Nuclear Chemistry and Technology,PL03195 Warsaw

Poly(ADP-ribose) is synthesized in cells following induction of DNA damage. We analyzed poly(ADP-ribose) metabolism in two closely related mouse lymphoma sublines L5178Y with differential radiosensitivity: radiation resistant LY-R and radiation sensitive LY-S cells, following exposure to 2 Gy of X-rays. NAD (the substrate for poly-(ADP-ribose) polymerase) and ADP-ribose polymers were purified using boronate affinity chromatography. Subsequently, NAD was analysed by HPLC and polymers by high-resolution polyacrylamide gel electrophoresis. NAD depletion following X-ray irradiation was observed in LY-S cells, reflecting dynamic changes in poly(ADP-ribose) polymerase and -glycohydrolase activities. On the other hand, LY-R cells showed no NAD depletion and no dramatic alteration in polymer size distribution.

S05-42

In vitro DNA Replication in Yeast Nuclear Extracts

D. Braguglia, P. Pasero and S.M. Gasser,
ISREC, Ch. des Boveresses 155, CH - 1066 Epalinges.

The yeast *S.cerevisiae* provides a powerful system for studying DNA replication, being the only eukaryotic organism in which origins of replication have been characterised in detail. In addition, a large number of mutants known to be involved both in DNA synthesis and the initiation of DNA replication have been isolated.

A soluble *in vitro* DNA replication system based on yeast nuclear extracts is presented. By using two dimensional (2D) gel electrophoresis we have detected the presence of bidirectional migrating replication forks in extracts prepared from cells synchronised in S-phase but not in extracts from cells blocked in G1. CsCl density gradient indicate that the input plasmid DNA is semiconservatively replicated.

A role for DNA polymerase α in this DNA synthesis was demonstrated by the complete inhibition of the reaction by anti-DNA polα antibodies and by using extracts prepared from temperature sensitive (ts) primase mutants (*pri1-1* and *pri 2-1*). The involvement of DNA pol ∂ was monitored with extracts prepared from *cdc2-2* ts cells. No mutant tested was able to support DNA replication at the non permissive temperature (37°C) as shown by 2D gel electrophoresis. Complementation between *pri2-1* and *cdc2-2* was observed at 37°C, while only S-phase extracts but not G1 extracts were able to restore replication in the *cdc2-2* mutant extracts.

A role for the involvement of CDC6 in the replication machinery was suggested by using cdc6-1 ts cells. By 2D gel electrophoresis no replication intermediates were detected at the non permissive temperature.

Intact yeast nuclei were assayed for replication in soluble nuclear extracts by incorporation of biotinilated nuleotides into nascent DNA and the sites of incorporation were detected by confocal microscopy. The observed DNA synthesis is under the cell cycle control and replicated nuclei display a spotted pattern suggesting that replication origins are organized into replication foci within the yeast nucleus.

We are currently investigating the effects of *mcm/cdc46* mutants in the observed replication pattern using complementation assays in vitro. The functional relationship between the origin recognition complex (ORC) and the *mcm* proteins will also be discussed.

S05-43

REGULATION OF GENE EXPRESSION IN THE DEVELOPING EXOCRINE PANCREAS OF RODENTS.

A. Krapp, M. Knöfler, L. Sommer, O. Hagenbüchle and P.K. Wellauer
I.S.R.E.C., Epalinges.

Exocrine pancreas-specific gene expression is under control of transcription factor PTF1, a cell-specific DNA-binding activity. PTF1 is a heterooligomer containing three distinct bHLH protein subunits. Two of them, p64 and p48, bind as a heterodimer to a bipartite DNA motif. p48 recognizes the canonical binding site for bHLH proteins CANNTG. p64, which is closely related to murine bHLH protein ALF1, recognizes the sequence TGGGA. The p75 subunit, which is the rat counterpart of human bHLH protein E12, does not contact the DNA but is required for the transport of the factor into the nucleus since it provides the factor with an active nuclear localization signal.
The p48 DNA-binding subunit is a novel protein related to other developmental regulators. It is essential for pancreas-specific transcription since exocrine pancreatic cells expressing antisense p48 RNA are impaired in specific functions. p48 mRNA accumulates not only in the exocrine pancreas but also in the skin epidermis, a tissue that lacks PTF1. In the developing pancreas, p48 mRNA synthesis preceeds the onset of PTF1 binding activity suggesting that the protein may have additional functions early during development. To address this question we have generated a p48 null mutant mouse.

S05-44

MOLECULAR KARYOTYPING OF THE DEVELOPMENTALLY REGULATED *LEISHMANIA* HISTONE H1-LIKE GENE SW3

Sabina Belli, Pascal Cousin*, Phil Shaw* & Nicolas Fasel, Biochem. Inst., UNIL, Epalinges; Dept. Oncology & Experimental Pathology, CHUV, Lausanne

Leishmaniasis is a parasitic disease which manifests itself as deforming yet self-healing cutaneous and mucosal lesions, to more severe, lethal forms affecting visceral organs. During the bloodmeal of a sandfly, *Leishmania* promastigotes infect host macrophages, where they differentiate into amastigotes, and multiply. In order to understand the mechanisms underlying gene expression in promastigote versus amastigote stages of *Leishmania major*, we characterized a histone H1-like gene SW3. SW3 has elevated levels of RNA expression in amastigotes versus promastigotes. PCR amplification of genomic DNA using defined primers gives rise to three bands (A1, A2, A3), with a size difference of about 60 bp. These DNA fragments are recognized by Southern blotting using SW3 cDNA as probe, show sequence homology to SW3 and give rise to the same RNA expression pattern as SW3. Molecular karyotyping of these genes reveal that they are co-localized to a 1.2 Mbp DNA fragment. It could be speculated that these genes belong to a family of SW3/Histone H1-like proteins.

S05-45

TISSUE-SPECIFIC AND UBIQUITOUS FACTORS BINDING NEXT TO THE GLUCOCORTICOID RECEPTOR MODULATE TRANSCRIPTION FROM THE MOUSE MAMMARY TUMOR VIRUS PROMOTER

C. Cavin and E. Buetti, ISREC, 1066 Epalinges.

Steroid hormones complexed with their receptors play an essential role in the regulation of MMTV transcription. However, the need for additional tissue-specific regulatory factors is suggested by the lack of virus expression in liver, where glucocorticoid receptors are highly abundant and by the tissue-specific transcription of reporter genes linked to an MMTV LTR in transgenic mice. In this study we characterized two regulatory elements, DRa and DRc, which, together with the distal glucocorticoid receptor binding site (DRb) increased transcription from the MMTV promoter in permissive cells. This was demonstrated by transfection of these sequences (DRa, DRb and DRc) in different combinations with the natural MMTV promoter in mouse fibroblasts and mammary epithelial cells, followed by quantitative S1 nuclease mapping of the transcripts. We further showed by DNaseI footprinting, methylation interference and gel retardation assays with various nuclear extracts from permissive or non-permissive tissues and cell lines that the factor(s) binding to the DRa site are distinct and tissu-specific whereas those binding to DRc are ubiquitous.

S05-46

Closely related species of filamentous fungi with different optimal growth temperatures do not display the same heat shock protein pattern

Oberson, J. & Canevascini, G., Institut de Biologie Végétale, Rue Albert-Gockel 3, CH-1700 Fribourg

Thermophilic organisms, either procaryotes or eucaryotes, have been known to exist for nearly a century. However, most of the significant studies on thermophily have been carried out during the last thirty years mainly on bacteria. Among fungi, the large genus *Chaetomium* comprises a few species that are definitely thermophilic and others that are thermo-tolerant. The overall similarity between the mesophilic and the thermophilic species is so striking that the ability to grow at higher temperatures (40 - 50 °C) could possibly be linked to a relatively small number of genetic determinants. In order to see if such a determinant could involve the expression of specific heat shock proteins (HSP), a comparison was made of the SDS-PAGE HSP pattern from an *in-vivo* radiolabelled culture of a typical thermophile (*C. thermophile* growing at 47 °C) with those from organisms growing at lower temperatures (*C. cellulolyticum* and *C. brasiliense* at 39 °C and 35 °C, resp.). To obtain comparable results, care was taken to grow the different strains at temperatures inducing the same transient (60 min) expression of HSP. Although it was always possible to distinguish the induction of at least 15 HSP, autoradiograms did not reveal clearcut differences for members of HSP families of high MW (i. e. 60, 70, 90 and 100 kD) on either 1 or 2D-PAGE. On the other hand, several differences were clearly noted concerning proteins of lower MW, among others, a neutral 32 kD protein and a 45 kD group found only in *C. brasiliense* (a typical mesophile) and groups of acidic proteins (55, 40.5 and 20 kD) which seemed to be specific to *C. thermophile*.

S05-47

***TNR3* OF *S. POMBE* ENCODES THE THIAMINE PYROPHOSPHOKINASE**

A. Zurlinden, H. Fankhauser and M. E. Schweingruber; Institute for General Microbiology, Baltzerstr.4, 3012 Bern

In *S. pombe*, thiamine is a potent regulator (repressor) of gene expression for genes involved in thiamine metabolism. *Tnr3* mutants reveal no or an impaired repression of thiamine regulated genes in the presence of thiamine, indicating that *tnr3* encodes a regulatory protein. It's DNA sequence shows a high homology with *THI80* of *S. cerevisiae*, which encodes the thiamine pyrophosphokinase (TPK). A *tnr3* disruption is lethal. Measurements of TPK activity in *tnr3* mutants and strains overexpressing the *tnr3* gene showed a significantly reduced or an increased activity, showing that *tnr3* indeed encodes the TPK. This indicates that TPK is essential and acts as a metabolic regulator for thiamine regulated genes. The intracellular level of thiamine diphosphate is probably the regulatory signal for gene regulation.
S. pombe TPK was also roughly enzymatically characterized.

S05-48

C. elegans homeobox-13 against cellular and embryonic haphazard in A-P asymmetry

C. Wittmann, K. Brunschwig, H. Tobler and F. Müller
Institute of zoology, University of Fribourg, Pérolles, Fribourg, Switzerland
email: claudia.wittmann@unifr.ch

The *C. elegans* embryo develops from six founder cells whose lineage history is almost completely invariant from one animal to the other. Using specific immunolocalization and a β-galactosidase reporter system, we have localized the *ceh-13* protein product as early as in the 26-cell stage in cells from two of these lineages. All of these cells are the posterior partners of the last division, suggesting that *ceh-13* is not a lineage determinant but rather that it might participate in *cellular* antero-posterior asymmetry.

At the end of gastrulation, the *ceh-13* protein is located in many cells of the anterior region of the embryo and becomes restricted to motoneurons during late larval and adult stages. A possible contribution of *ceh-13* to the *embryonic* antero-posterior axis is supported by phylogenetic analysis of its sequence. We indeed could show that *ceh-13* is the orthologue of the *labial*-like genes of the HOM-C and Hox clusters in *D. melanogaster* and vertebrates, respectively, which mediate time- and position-specific informations during embryonic development.

Analysis of the phenotype of a *ceh-13* mutant allele will help dissecting the putative functions of *ceh-13* (see the abstract of K. Brunschwig et al.).

S05-49

CLONING AND CHARACTERIZATION OF A GENE CODING FOR A PUTATIVE GTP-BINDING PROTEIN LOCATED CLOSE TO A CHROMOSOME BREAKAGE REGION DURING CHROMATIN DIMINUTION IN *ASCARIS LUMBRICOIDES*

Huang Y. J., Tobler H. and Müller F., Institute of Zoology, University of Fribourg, Pérolles, CH-1700 Fribourg

Ascaris lumbricoides and a few other nematodes undergo chromatin diminution in the somatic precursor cells of the early embryo, confirming the segregation and independent development of the germ and somatic cell lineages in those nematodes. During this process about 25% of the total *Ascaris* germ line genome is expelled from presomatic cells. Chromosomal fragmentation occurs within specific chromosomal breakage regions (CBRs) and is followed by the addition of repeats of the telomeric sequence TTAGGC. One of these CBRs (CBR1) was analyzed in detail. A non-eliminated gene located near CBR1 which encodes a putative GTP-binding protein (*A-gbp*) has been identified. A gene homologous to *A-gbp* has also been isolated from *C. elegans* (*Ce-gbp*). *Ce-gbp* is also located near a telomere: It is the last mapped gene on the left end of chromosome V. A high degree of sequence conservation of this gene between the two nematode species and man (a corresponding cDNA clone was identified in a human cDNA library) suggests an important function. We are now trying to use genetic methods to determine its function in *C. elegans* and to test whether its telomeric localization in both nematode species could have functional importance. *A-gbp* provides us with the opportunity to check whether the chromosomal rearrangements associated with chromatin diminution have an influence on the expression of nearby located genes. Northern blot analyses with the *A-gbp* probe revealed that transcripts are present in all developmental stages. This suggests that the new telomere formation during chromatin diminution does not influence gene expression.

S05-50

TELOMERASES IN NEMATODES

Magnenat L., Nilsen* T. W., Tobler H. and Müller F.
Institute of Zoology, University of Fribourg, Pérolles, CH-1700 Fribourg. email: Laurent.Magnenat@unifr.ch
*School of Medicine, C.W.R.U., Cleveland, Ohio 44106, USA

During the process of chromatin diminution in *Ascaris lumbricoides*, most but not all of the germ line telomeres are removed and new somatic telomeres become attached to the chromosomal fragmentation sites, probably by a strong telomerase activity.
In vitro extracts from chromatin eliminating developmental stages are thus expected to be well suited for the isolation of the telomerase protein(s) and its RNA component (or other co-factors). Therefore, *A. lumbricoides* extracts from 4-8 cell stages were established and tested for *in vitro* telomerase activity. In parallel, we are developing cell-free extracts of *C. elegans*.
The nematodes *A. lumbricoides* and *C. elegans* represent an excellent system for the analysis of telomerase. Its isolation might provide an entry point towards genetical analysis of telomere function in *C. elegans* and will reveal its importance for the development of multicellular organisms.

S05-51

DEVELOPMENTALLY PROGRAMMED BREAKAGE AND HEALING OF CHROMOSOMES IN ASCARIS LUMBRICOIDES

Stephan Jentsch, Heinz Tobler and Fritz Müller
Institute of Zoology, University of Fribourg, CH-1700 Fribourg

In the course of the early embryonic development, the chromosomes of the parasitic nematode *A. lumbricoides* and some other nematodes undergo chromatin diminution in all presomatic cells. During this process, which occurs between the third and the fifth embryonic cleavage divisions in five different blastomeres, the heterochromatic termini of the chromosomes are cut off and become degraded in the cytoplasm. The truncated chromosomes are healed by the addition of newly formed telomere. New telomere addition takes place within a well defined region, called CBR (chromosomal breakage region), of which many different copies are present in the *Ascaris* genome.
A cloned CBR (CBR1) was further analyzed in detail: A PCR experiment revealed, that the distribution of the telomere addition sites within CBR1 is random. No sequence homologies or secondary structures are conserved between the different telomere addition sites. The presence of 1-4 ambiguous nucleotides at the junction between the CBR1 sequences and the newly added telomeric repeats strongly suggests that telomere addition is mediated by a telomerase activity ("healing process").
An identical PCR experiment was performed for the eliminated material flanking CBR-1 with total DNA isolated from synchronized 4-cell embryos. These experiment revealed that telomeres are not only added to the ends of the truncated somatic chromosomes, but also to the ends of the eliminated chromatin.

S05-52

THE *LABIAL*-LIKE GENE IN *C.ELEGANS*

Brunschwig K., Wittmann C., Plasterk R.H.A.*, Tobler H.,Müller F.
Institute of Zoology, University of Fribourg, 1700 Fribourg-CH
*Netherlands Cancer Institute, Amsterdam, The Netherlands

The *C. elegans* HOM-Cluster located on the third chromosome of the nematode consists at least of four Antennapedia-class homeobox genes: *lin-39*, *ceh-13*, *mab-5* and *egl-5*. Mutants of *lin-39*, *mab-5* and *egl-5* show defects in the specification of positional cell fate along the antero-posterior axis.
Preliminary data concerning the pattern of *ceh-13* expression (see the abstract of Wittmann et al.) suggests that the function of this gene is important in the development of the nematode. Since no mutant allele exists for *ceh-13* (a *labial*-like homologue), we have isolated a Tc1 insertion from a frozen *C. elegans* mutant bank and by PCR and sib-selection will isolate a deletion derivative created by the imprecise repair associated with excision of the Tc1 transposable element. In a parallel approach, we are performing complementation tests with a set of EMS induced lethal mutants which have been mapped to the region of *ceh-13* by the group of Dr. D. Baillie (Vancouver).

S05-53

CEC-1: A CHROMO DOMAIN CONTAINING PROTEIN IN *C. ELEGANS*

Agostoni, E., Hill, F., Wittmann, C. ,Tobler, H., and Müller, F.*
Institute of Zoology, University of Fribourg, CH -1700 Fribourg.
**Dept. of Haematology, University of Cambridge, UK.*

The chromo domain (Chromatin Organization Modifier) is a phylogenetically conserved sequence motif of 48 amino acids (aa) length, which has been first detected as a region of homology between the two *Drosophila* proteins Polycomb (Pc) and Hp1. Both proteins are essential for viability, associated with heterochromatin and gene silencing. Pc is an important repressor protein, necessary to maintain the correct spatial expression pattern of developmental regulatory genes, among them the homeotic genes of the ANT-C and BX-C complexes. Hp1, on the other hand, is a constitutive component of the *Drosophila* heterochromatin and is encoded by Su(var)205, a suppressor of position effect variegation with recessive lethality . The particular function of the chromo domain is not yet understood, but it seems to be important for protein-protein interactions in chromatin associated complexes. Here we show that *C. elegans* contains at least three different chromobox containing genes. Length and structure of these putative chromo domain proteins are similar to those of the chromo domain containing proteins of other species. The expression pattern of one of the chromo domain proteins of *C. elegans* (Cec-1) was further studied . Cec-1 is expressed in the nuclei of somatic cells from the 80-100 stage cell embryo until the adult, while no expression is observed in the germ cells and in the early embryo. To learn more about the function of Cec-1 we are currently isolating and studying a Tc1 insertion of its gene.

S05-54

TELOMERIC REPEATS ARE SUFFICIENT FOR CHROMOSOME STABILITY IN*CAENORHABDITIS ELEGANS*

C. Wicky, M. Zetka, N. Lauper, H. Tobler and F. Müller, Inst. of Zoology, University of Fribourg, Pérolles, CH-1700 Fribourg.

The telomeric sequence located at the ends of the six *C. elegans* chromosomes consist of the tandemly repeated hexamer, TTAGGC. In order to analyze the organization and function of telomeres in the nematode, a library selecting for terminal sequences was constructed and screened for recombinants containing the TTAGGC repeat. Twelve unique clones have now been isolated and tested positive for *Bal*31 sensitivity, indicating that they represent the twelve true native telomeres. Two of these clones have been mapped to their chromosome of origin by PCR deficiency mapping and by analyzing unique subtelomeric sequences. In addition, the breakpoint of a terminal deficiency of the *X* chromosome (*me8*) has been cloned and analyzed, revealing that the addition of telomeric repeats was sufficient to stabilize the broken end.

To further test the role of telomeric repeats in chromosome stability, linear constructs capped with telomeric repeats are being injected into hermaphrodites to generate transgenic animals. The stabiltiy of these linear plasmids will be determined and compared to the stability of constructs lacking TTAGGC repeats at the end.

S05-55

Linkage Between Stable Repression and DNA Replication: The gene *cramped* of *Drosopila melanogaster*

Yutaka Yamamoto, Markus Affolter, & Walter J Gehring, Dept. of Cell Biology, Biozentrum, University of Basel, CH-4056 Basel

In *Drosophila* a class of genes called Polycomb-group genes has been identified, which is required for maintenance of homeotic gene activity. After the initial establishment of the expression pattern of homeotic genes, the Polycomb-group genes are involved in the stable transcriptional repression in those cells in which a given homeotic gene is not expressed. We have cloned a new gene belonging to this group, which is designated as *cramped (crm*, formerly called *sparse arista* or *subarista). Crm* males show both posterior to anterior transformations (extra sex combs on the second and third legs) as well as distal to proximal transformations (extra sex combs on the second and third tarsal segments). Antibody staining of early embryos revelaed that the CRM protein is localized in the nucleus in a cell cycle-dependent manner. During interphase CRM is located in the nucleus, but it is translocated to the cytoplasma at the time of chromosome condensation and re-enters the nucleus after telophase. We found that proliferating cell nuclear antigen (PCNA), which is known to be involved in DNA replication, shows a similar cell cycle-dependent behavior and appears to co-localize with CRM in salivary gland nuclei. Furthermore, flies carrying certain mtuations in the *mus (mutagen sensitive) 209* gene, which is the homologue of the mammalian PCNA gene, have *crm* like phenotypes. These observations suggest that *crm* function provides a link between stable transcriptional repression and DNA replication.

S05-56

HOMEODOMAIN BINDING SITES FROM MOUSE Hox GENES FUNCTION AS ENHANCERS IN *Drosophila* EMBRYOS AND IMAGINAL DISCS. Liam Keegan, Theo Haerry and W.J. Gehring. Department of Cell Biology, Basel University Biocentre, Klingelbergstrasse 70, Basel CH4056

The basis for Antennapedia target specificity *in vivo* is not known. Antennapedia binds as a monomer *in vitro* to short sequences in which less than six base pairs are conserved. Oligomerizing binding sites does not produce an enhancer that can direct a meaningful expression pattern. Very few enhancers have been identified that are directly bound by the homeotic proteins. Functional target enhancers for homeotic proteins may have special structures, with particular arrangements of homeodomain binding sites and/or binding sites for additional cofactors. As so many aspects of homeotic protein expression and function are conserved between flies and vertebrates there could also be some conservation of target enhancer structures.

An interesting way to identify homeodomain binding site clusters that may be very highly active or evolutionarily conserved is to study the enhancer activity of sequences from vertebrate *Hox* genes in *Drosophila.* We have found that the intron of the mouse *Hoxa4* gene directs very strong expression in the embryo and in the leg imaginal discs in *Drosophila.* Expression in the antennal disc is activated in response to ectopic expression of Antennapedia, which causes an antenna to leg transformation, and also by Ubx or AbdA proteins but not by other homeotic proteins. The proteins that activate share particular specificity-determining residues in the amino terminal arm of the homeodomain that are not shared by the other proteins. We are currently analyzing whether the interaction of Antennapedia and Ubx proteins with these sites are direct *in vivo* by using the altered specificity approach.

This work suggests that functional target sequences have some features that are conserved all the way from mice to *Drosophila.*

S05-57

THE *DROSOPHILA* MODIFIER OF POSITION EFFECT VARIEGATION *E-VAR(3)164* ENCODES THE TRANSCRIPTIONAL ACTIVATOR E2F1

Seum, C., Pauli, D. and Spierer, P.
Department of zoology and animal biology, University of Geneva, 154 route de Malagnou, CH-1224 Chêne-Bougeries

In a collection of transposon-induced dominant modifiers of position-effect variegation generated by G. Reuter (Halle), we found that the *Evar(3)164* mutant resulted from an insertion of the transposon in a large intron, upstream of the translation start, of the *Drosophila* homolog of the transcriptional activator E2F1. In the control of the cell cycle, this factor transactivates late G1 genes, which are required for entry into the S phase.The gene maps at 93E on polytene chromosomes. Northern blot analysis shows three sizes of transcripts at 3.6, 4.2, and 4.7 kb. In homozygous mutant flies, the amounts of each transcript is strongly reduced. Three classes of cDNA clones have been isolated from an embryonic library (4-8 hours). The cDNAs differ mostly in their 3' non coding region.

S05-58

***E-VAR(3)64E*, AN ENHANCER OF POSITION EFFECT VARIEGATION IN *DROSOPHILA* WITH AN APPARENT PATERNAL IMPRINTING-LIKE PHENOTYPE**

Henchoz S., De Rubertis F., Pauli D., *Reuter G. and P. Spierer.
Department of zoology and animal biology, University of Geneva, 154 route de Malagnou, CH-1224 Chêne-Bougeries, and *Institut fur Genetik, Martin-Luther-Universität, D-06108 Halle/S.

Position-effect variegation is the mosaic expression of a euchromatic gene brought at vicinity of heterochromatin by a rearrangement. The *Evar(3)64E* mutant results from an insertion of the P transposon at 64E on the polytene chromosome map. Genomic DNA was isolated by plasmid rescue. A transcript was detected, and corresponding cDNA clones isolated from an embryonic library. Sequencing reveals in the deduced protein a possible homology with the C-terminal domain of ubiquitin, a region implicated in transient modification of some histones during mitosis. Interestingly, the enhancer phenotype persists in males, even in the absence of the mutated gene, when their Y chromosome originates from a male carrying the autosomal enhancer mutation.

S05-59

ANALYSIS OF THE *TRITHORAX-LIKE* LOCUS, THE GENE ENCODING THE *DROSOPHILA* GAGA BINDING FACTOR

Farkas, G.[1], Gyurkovics, H.[2], Gausz, J.[2], and Karch,[1] F. [1]Dept. of Zoology and Animal Biology, University of Geneva, 154 rte de Malagnou, 1224 Geneva, Switzerland. [2]Inst. of Genetics, BRC, POBox 521, 6701 Szeged, Hungary.

Genetic analysis in *Drosophila* has led to the discovery of two classes of genes, the regulators of homeotic genes and the modifiers of position-effect variegation (PEV), which seem to comprise good candidates for encoding some of the factors regulating chromatin functions. We have identified a locus, the *Trithorax-like* gene (*Trl*) which is required for the normal expression of the homeotic genes and is a modifier of PEV. We found that *Trl* encodes the GAGA factor which has been shown to be involved in the formation of an accessible chromatin structure at promoter sequences. Our data indicate that the activity of GAGA is not restricted to promoters. We will also discuss the effects of the nearly complete absence of GAGA factor in early development.

S05-60

FUNCTIONAL DOMAINS OF THE HEAVY METAL-RESPONSIVE TRANSCRIPTION REGULATOR MTF-1

Hans-Peter Müller, Freddy Radtke, Oleg Georgiev, Enrico Brugnera, Karl-Heinz Müller and Walter Schaffner, Institut für Molekularbiologie II der Universität Zürich, CH 8057 Zürich

We have previously cloned a zinc finger factor (MTF-1) that binds specifically to heavy metal-responsive DNA sequence elements in the promoters of metallothionein genes, and shown that this factor is essential for both basal and heavy metal-induced transcription. The C-terminal part of MTF-1 downstream of the zinc fingers harbors three different transactivation domains, namely an acidic domain, a proline-rich domain and a domain rich in serines and threonines. All of these domains can function in concert or independently when fused to the DNA binding domain of the yeast factor GAL4. However, transcriptional activity is constitutive, i.e. transcription of a GAL4-driven reporter gene is not induced by heavy metals. In search of the region responsible for metal induction, deletion mutations of mouse MTF-1 and chimera between human and mouse MTF-1 were tested with a reporter gene driven by a metal-responsive promoter. The zinc fingers, that are dependent on zinc for efficient DNA binding, can confer some zinc responsive transcription by themselves. The C terminal region was identified to be required for both transcription efficiency and full zinc responsiveness. Thus the activity of MTF-1 results from a complex interplay of different functional domains, not all of which seem amenable to a reductionist dissection approach.

S05-61

CHARACTERIZATION OF BOVINE HERPESVIRUS 1 IMMEDIATE-EARLY PROTEINS BICP0, BICP4, BICP22, *CIRC*, AND EARLY PROTEIN BICP27

René Köppel,[1] Mahender Singh, Cornel Fraefel, Martin Schwyzer, and Mathias Ackermann

Institute of Virology, Faculty of Veterinary Medicine, University of Zürich, CH-8057 Zürich, Switzerland

Bovine herpesvirus 1 (BHV1) specifies three immediate-early (IE) proteins named BICP0, BICP4, and BICP22. A fourth BHV1 IE protein designated *circ* is encoded by a transcript spanning both ends of the viral genome and has counterparts in EHV1 ORF3 and VZV ORF2 but not in HSV1. Finally, a BHV1 protein named BICP27 is expressed with early kinetics, whereas its HSV1 counterpart is an IE protein. Based on the nucleotide sequence of the entire IE region we raised antisera against synthetic oligopeptides and used them to characterize all five proteins by Western blotting, immunofluorescence (IF) and immunoadsorbent staining. BICP0 (97 kDa)was localized in the nuclei and by IF exhibited a punctate pattern. BICP4 (180 kDa) was also localized in the nuclei but had a clustered appearance. BICP22 (50 kDa) and BICP27 (50 kDa) exhibited both uniform localization in the nucleus. The only protein with cytoplasmic localization was *circ* (34 kDa) which was also shown to be associated with virions and to be myristylated. A viable BHV1 recombinant containing a β-gal-substitution in the *circ* gene was isolated, indicating that *circ* is non-essential for virus replication in cell culture. The nuclear localization of BICP0, BICP4 and BICP22 was expected from their function as activators and/or repressors of viral gene expression; the functions of the other two proteins have not yet been defined. BICP0, BICP27 and *circ* have been expressed in recombinant baculoviruses.

S05-62

BOB-1, A B-CELL COACTIVATOR OF OCTAMER BINDING TRANSCRIPTION FACTORS

Matthias Gstaiger, Lea Knoepfel, Oleg Georgiev, Antonia Manova, Walter Schaffner and Christopher Hovens°

Institut für Molekularbiologie II der Universität Zürich, CH 8057 Zürich
° present adress: Institut für Medizinische Virologie der Universität Zürich Gloriastrasse 30, CH-8028 Zürich, Switzerland

The octamer motif (ATGCAAAT) paradoxically, plays a central role in mediating the activity of both B-cell specific and ubiquitous promoters. It has been widely assumed that the predominantly lymphoid restricted octamer binding factor Oct-2 mediates tissue specific promoter activity, whereas the ubiquitously expressed Oct-1 mediates general promoter activity. However, this view has been challenged by recent observations. Here we use a modified yeast one hybrid assay to isolate a B-cell factor, BOB-1, that associates with either Oct-2 or Oct-1. In transfection experiments this factor boosted Oct-1 mediated promoter activity, and to a lesser extent that of Oct-2. This coactivation was strictly dependent on the specific interaction with Oct-1 or Oct-2 since deletion of the octamer motif abolished coactivation. We conclude that BOB-1 may represent a novel tissue-specific transcriptional coactivator that may convert a ubiquitously expressed transcription factor to a cell type specific activator.

S05-63

The GA-rich motif counteracts Neuronal Oct factor repression of Oct-1/VP16-mediated transcription at HSV TAATGARAT sequences.

Philippe Douville, Michael Hagmann, Oleg Georgiev and Walter Schaffner
Institute for Molecular Biology II, University of Zürich, Zürich, Switzerland

In permissive cells HSV produces lytic infections by transcribing its immediate early (IE) genes through corecruitment of the virion protein VP16 (Vmw65) and the cellular factor Oct-1 onto upstream TAATGARAT sequences. In neurons, however, immediate early gene transcription is blocked. Our efforts are focused towards determining if specific neuronal factors are involved in regulating HSV IE gene transcription and whether they may be involved in HSV latency. Indeed, neuronal Oct factors (N-Oct2 and N-Oct3) bind to TAATGARAT sequences from the viral ICP0 and ICP4 promoters. Our results using *in vivo* transcription assays indicate that N-Oct2 (Brn1) or N-Oct3 (Brn2) can repress Oct-1/VP16-mediated transcription from isolated TAATGARAT elements but not from the natural ICP4 promoter suggesting that additional sequences in the promoter participate in the VP16- induced response. Constructs containing the GA-rich motif upstream from the TAATGARAT sequence are not repressed to the same extent by NOct factors perhaps due to cooperativity between GA-binding protein (GABP) and the Oct-1/VP16 complex. The molecular nature of this cooperativity is currently under investigation.

S05-64

THE POLYCOMB RESPONSE ELEMENT ESTABLISHES A REPRESSIVE CHROMATIN STATE AT THE Ubx GENE.

PIRROTTA,V., RASTELLI,L., TATOUT,C. & VALENTI,P.
Dépt. de Biologie Animale, University of Geneva.

The Drosophila Ubx gene is regulated by segmentation genes during early embryonic development. When these regulators cease being expressed, the maintenance of the expression pattern is guaranteed by a complex formed by the products of the Polycomb Group of genes (Pc-G) at the Polycomb Response Element (PRE). The PRE complex establishes a repressive chromatin state that affects a large chromatin region in cells in which the Ubx gene was inactive at the time the complex was established. The PRE contains several target sequences for different Pc-G proteins as well as for proteins such as zeste, GAGA factor and trithorax which stimulate expression in cells in which the repressive complex is not established. In vivo, the PRE contains a set of DNase hypersensitive sites flanked by GAGA factor binding sites. The PRE is flanked by A+T rich sequences which behave as SARs but are not essential for PRE function. We are examining the response of these chromatin features to mutations in different components of the Pc-G and trithorax group of genes.

S05-65

Formation and Repair of UV induced DNA Lesions in Chromatin

R. Wellinger, S. Tanaka and F. Thoma, Institut für Zellbiologie, ETH-Hönggerberg, CH-8093 Zürich, Tel. 01-6333344, Fax 01-6331069

Plasmid DNAs with a single cyclobutane thymine dimer (CPD-TT) or a 6-4 thymine-thymine photoproduct (6-4 PP-TT) were used as a substrate for primer extension by Taq polymerase. The plasmids were linearized with Hind III and primer extension was done using an endlabelled primer and 30 cycles of linear amplification. To test how efficient the Taq polymerase is blocked at CPDs and 6-4 PP, the CPD was digested with T4EndoV and the 6-4 PP cleaved with piperidine. We show that Taq DNA polymerase is completely blocked at CPD-TT and 6-4 PP-TT. This assay is currently used to correlate chromatin structure with DNA damage formation and repair by the nucleotide excision pathway (NER) in a yeast minichromosome TRURAP after UV irradiation. Preliminary data indicate differential repair in the nonnucleosomal and nucleosomal region of the origin of replication (ARS1).

Signal transduction in animal cells

S06-01

Structure and function of the Pleckstrin Homology (PH) domain

Matti Saraste, European Molecular Biology Laboratory, Postfach 102209, D-69012 Heidelberg, Germany.

The PH domain is a 100-residue structural motif that is found in many signalling and cytoskeletal proteins. The structure of several PH domains have been determined by NMR and X-ray techniques. The core of these structures is a similar antiparallel β-sheet consisting of seven strands with a long C-terminal α-helix. PH domains appear to be highly charged, and it is possible that electrostatic polarization is important for function. Previous experimental data have suggested that the PH domain binds phosphatidylinositol derivatives. We have studied the binding of PIP2 and related molecules to the spectrin PH domain. The head group of PIP2 as well as IP3 bind to the positively charged end of the domain with affinities in the micromolar range. Biological implications of this finding will be discussed.

S06-02

REGULATION AND FUNCTION OF RAS PROTEINS: CONNECTIONS WITH LIPID KINASES

Julian Downward, Imperial Cancer Research Fund 44 Lincoln's Inn Fields, London WC2A 3PX, U.K.

Ras proteins are key regulators of normal and malignant cell proliferation. A number of likely effector enzymes acting downstream of Ras have now been identified. Ras interacts directly with the Raf serine/threonine in a GTP dependent manner both *in vitro* and *in vivo*, but it has not been possible to show an effect of this binding on the kinase activity of Raf. Recent work on the role of phosphatidylinositol 3' kinase in mediating the Ras response will be discussed. At least four different isoforms of phosphatidylinositol 3' kinase interact with Ras in a GTP-dependent manner through the Ras effector site. The interaction involves a conserved region of p110 C-terminal to the p85 binding site. The interaction of Ras with phosphatidylinositol 3' kinase may be involved in the regulation of cytoskeletal structure.

S06-03

EFFECTS OF WORTMANNIN ON SIGNALLING PATHWAYS

Proud, C., Welsh, G., Flynn, A., Foulstone, E., Redpath, N., Moule, K., Avison, M., Edgell, N. and Denton, R.

Department of Biochemistry, University of Bristol, Bristol, BS8 1TD, United Kingdom

Wortmannin is a potent inhibitor of phosphatidylinositol 3-kinase, which is implicated in cell signalling due to its association with receptor tyrosine kinases or their substrates. We have examined the effects of wortmannin on a range of signalling pathways, particularly those involved in the regulation of biosynthetic processes, in Chinese hamster ovary cells, adipocytes and other cell-types. At 100nM, wortmannin blocks the activation of MAP kinase and its substrate, $p90^{rsk}$, as well as p70 S6 kinase, by insulin, serum and certain other agents, but has little effect on stimulation by phorbol ester. Wortmannin also blocks the inactivation of glycogen synthase kinase-3 as well as the activation of glycogen synthase and acetyl-CoA carboxylase by insulin in fat cells. We have further examined the effects of wortmannin on the phosphorylation of several translation factors in response to insulin and other agents. For example, wortmannin blocks the dephosphorylation of elongation factor-2 and the inactivation of eEF-2 kinase induced by insulin or serum. The data will be discussed in the light of our current perception of the roles of PI 3-kinase and MAP kinase in regulating these and other proteins.

S06-04

punt ENCODES THE *DROSOPHILA* HOMOLOG OF THE MAMMALIAN ACTIVIN RECEPTOR

E. Ruberte[1], T. Mary[2], D. Nellen[2], M. Affolter[1] and K. Basler[2]
[1]Biozentrum der Universität, Klingelbergstr. 70, CH-4056 Basel
[2]Zoologisches Institut, Universität Zürich, Winterthurerstr. 190, CH-8057 Zürich

Communication among cells is a key process in the development of multicellular organisms. Among the signaling systems involved in cell communication are those mediated by the members of the transforming growth factor-β (TGF-β) superfamily of secreted polypeptides. These secreted proteins are involved in many developmental events in all organisms where they have been identified. Multiple type I and II transmembrane receptors for ligands of the TGF-β family have been identified and have been shown to contain a cytoplasmic region with a serine/threonine kinase (STK) domain.
To analyse the function of STK receptors in cell-cell interaction in Drosophila, we have identified genes encoding different type I and II receptors. Here we show that *punt* codes for a type II receptor of the Drosophila signaling peptide *decapentaplegic (dpp)*, a member of the TGF-β superfamily. The patterning defects observed in *punt* embryos mimick those previously described for mutations in the type I *dpp* receptor *thick veins (tkv)*. We show that both the type I *(tkv)* and type II *(punt)* receptors are essential for the transduction of the *dpp* signal during Drosophila development. Therefore, there is no functional redundancy between *dpp* type I and type II receptors *in vivo*.

S06-05

COLONY-STIMULATING FACTOR-1 AND *c-fms* EXPRESSION DURING TOOTH ERUPTION

Sommer B, Hofstetter W, Wetterwald A, Bickel M. Department of Removable Prosthodontics and Institute of Pathophysiology, University of Bern.

The developing tooth germ is surrounded by bone. Resorption and remodelling of this bone is essential for tooth eruption. Bone resorbing osteoclasts require colony-stimulating factor-1 (CSF-1) as a growth factor. The aim of this study was to investigate whether the expression of CSF-1 and of its receptor, the proto-oncogene *c-fms*, is modulated during molar tooth eruption in mice. At all ages examined (4 to 14 days), CSF-1 transcripts were detected in bone cells, presumably osteoblasts and osteoblastic precursors. No CSF-1 transcripts were detected in cells from the tooth organ, however. Transcripts encoding *c-fms* were expressed by osteoclasts, and by tissue macrophages of the dental pulp and the surrounding soft tissues. The distribution of *c-fms* transcripts in bone cells correlated well with the expression of tartrate-resistant acid phosphatase that was used as a marker of osteoclasts. The results show no appearent increase in the levels of CSF-1 and *c-fms* transcripts, or in osteoclast density.

Supported by grants SNF 31 365 06.92 and SSO 185.

S06-06

THE GLUTAMATE-EVOKED RELEASE OF ARACHIDONIC ACID FROM MOUSE CORTICAL NEURONS IS MODULATED BY A pH-SENSITIVE MEMBRANE PHOSPHOLIPASE A2

Nephi Stella[1,2], Luc Pellerin[1], and Pierre J. Magistretti[1] Institut de Physiologie[1], Universite de Lausanne & INSERM U114, College de France, Paris[2].

Excitatory synaptic transmission is associated with changes in both extracellular and intracellular pH. Using mouse cortical neurons in primary cultures, we studied the sensitivity of glutamate-evoked release of ^{3}H-arachidonic acid (*AA) to changes in extracellular pH (pHo) and related intracellular pH (pHi). As pHo was shifted from 7.2 to 7.8, the glutamate-evoked release of 3 *AA was enhanced by ~ 3 fold. The effect of alkaline pHo on the glutamate response was rapid, becoming significant within 2 min.. *AA release evoked by both NMDA and kainate was also enhanced by pHo alkalinisation. NMDA- and kainate-induced increase in free intracellular Ca^{++} was unaffected by changing pHo from 7.2 to 7.8, indicating that the receptor-induced Ca^{++} influx is not responsible for the pHo-sensitivity of the glutamate-evoked release of *AA. Membrane-bound phospholipase A2 (mPLA2) activity was stimulated by Ca^{++} in a pH dependent manner, increasing its activity as pH was shifted from 7.2 to 7.8. This pH-senisitvity profile corresponds to the pH profile of the glutamate-, NMDA- and kainate-evoked release of *AA. Taken together, these results indicate that the glutamate-evoked release of *AA may be mediated by a pH-sensitive mPLA2. Since excitatory neurotransmission mediated by glutamate results in both pHo and pHi changes and since AA enhances glutamatergic neurotransmission at both pre- and post-synaptic levels, the data reported here reveal a possible molecular mechanism whereby glutamate can modulate its own signalling efficacy in a pH-dependent manner by regulating the release of AA.

S06-07

SYNTHESIS OF MEMBRANE AND MATRIX BOUND COLONY- STIMULATING-FACTOR -1 (CSF-1) BY CULTURED OSTEOBLASTS. R. Felix, A. Wetterwald, M. Cecchini, H. Fleisch and W. Hofstetter, Pathophysiology, University of Bern, Murtenstrasse 35, 3010 Bern.

CSF-1 is the growth factor for the mononuclear phagocytic cells and the osteoclasts. It is synthesized as a secreted or a membrane associated molecule. The major part of the secreted form is posttranscriptionally modified by the attachment of a glycosaminoglycan chain, and this proteoglycan (PG) can be bound to the extracelluar matrix (ECM). Within this study we investigated the CSF-1 production by primary osteoblasts and the osteoblastic cell lines MC3T3-E1 and KS-4.

Layers of osteoblastic cells fixed with glutaraldehyde supported the proliferation of a macrophage cell line suggesting the presence of CSF-1 in the cell layer as a membrane and/or matrix associated form. Sequential treatment of the cell layer with 2 M NaCl to extract the matrix bound CSF-1, followed by trypsin digestion to release the membrane associated form, yielded two fractions containing low amounts of CSF-1 activity. The activities, either fixed on the cell layer or extracted, were neutralized with antiserum against CSF-1. To further characterize the synthesized CSF-1, cells were metabolically labeled with [^{35}S] met/cys or [^{35}S] SO_4^{2-}. Proteins in the supernatant were immunoprecipitated with an antiserum against rhCSF-1 and separated by PAGE. Bands with MW > 200kd, 200kd, 100kd and 50kd, shifting to lower MW under reducing conditions, were visualized by autoradiography. Treatment with chondroitin lyase ABC decreased the the size of the large MW monomer, proving the PG structure. Furthermore, CSF-1 was immunoprecipitated from the ECM and the trypsinized membrane fraction.

These data show: 1) Osteoblastic cells secrete CSF-1 mainly as the PG-form. 2) A small part of the secreted form is associated with the matrix. 3) A membrane associated form is synthesized.

S06-08

MONOCYTIC SURFACE ANTIGENS INVOLVED IN LPS-TRIGGERED CELLULAR ACTIVATION

A. Piani*, J.P. Hossle*, C.A. Siegrist#, S. Hüttner*, R.A. Seger* and R.P. Lauener*
*Division of Immunology/Hematology, University Children's Hospital, Zurich; #Department of Genetics and Microbiology, CMU, Geneva

Lipopolysaccharide (LPS), a cell wall component of gram-negative bacteria, causes septic shock. CD14, a glycosylphosphatidylinositol (GPI)-anchored monocytic antigen, has been shown to be a receptor for LPS. However, i) CD14-negative cells can respond to LPS, too. ii) it remains unclear how GPI-anchored molecules, lacking transmembrane and cytoplasmic regions, can transduce a signal upon engagement; one hypothesis is that GPI-linked molecules are associated with transmembrane proteins.
Molecules other than CD14 serving as additional receptors for LPS and/or involved in signal transduction upon engagement of CD14 have to be invoked to explain phagocyte activation by LPS. To identify such antigens we assessed the secretion of cytokines in response to LPS in cell lines lacking expression of antigens potentially involved in this process. Data will be presented.

S06-09

SIGNAL TRANSDUCTION THROUGH MEMBRANE BOUND IL-1

B. Spörri*, M. Bickel+, U.N. Wiesmann*; Laboratory of Metabolic Diseases* and Oral Cell Biology+, University of Bern.

Interleukin-1, originally described as a soluble proinflammatory cytokine, produced by monocytes/macrophages, was found to be constitutively expressed as a membrane associated, but not secreted protein in a variety of fibroblast cell strains. In the present studies we explored the function of membrane associated IL-1 and its regulation in human dermal fibroblasts (HDF). Stimulation of HDF with LPS, IL-1α or TNF-α, induced expression of IL-1ß and enhanced IL-6 mRNA steady state levels, as measured by quantitative RT-PCR. IL-1 protein could not be detected in the medium of stimulated HDF. Membrane bound IL-1 seems to play a functional role since incubation with monoclonal anti-human IL-1ß resulted in a dose dependent increase of IL-6 mRNA and IL-6 secretion, but not IL-1ß mRNA expression. This suggested that membrane associated IL-1 is engaged in signal transduction. Antibodies against IL-1 and/or soluble receptors, or IL-1 receptors being part of other cells, e.g. T cells, may thus trigger cellular responses in HDF, such as cytokine production.

+ supported by SNF 31 365 06.92

S06-10

Cultured CD 11a/CD 18 positive cells from bovine aorta

K. Spanel-Borowski, G. Herrmann, A.M. Ricken, and W.C. Davis*
Institute of Anatomy, University of Basel, Pestalozzistr. 20, CH-4056 Basel, Switzerland, *Department of Veterinary Microbiology and Pathology, Washington State University, Pullman WA 99164-7040, USA

During our study on cultured cytokeratin-positive and cytokeratin-negative endothelial cells from bovine aorta (Spanel-Borowski et al., Differentiation 57, 225, 1994), an unusual subpopulation was observed in postconfluent cultures of cytokeratin-negative endothelial cells. The unusual cells appeared on the apical side of the monolayer. Single cells proliferated and clusters of 2 to 6 cells developed within a hole of the monolayer. Multinucleated cells became apparent. By using immunocytochemistry without cell permeabilization, CD 11a/CD 18 integrins were preferentially detected at a front with lamellipodia and filipodia. After permeabilization with Triton X-100 CD 11a/CD 18 integrins were uniformly distributed. Globular actin was detected in the unusual cells with phalloidin-FITC. No ICAM-1 counter-receptors against CD 11a/CD 18 integrins were found. At the ultrastructural level, abundant electron light granules were seen, yet none of them were fused with the plasma membrane. Some Weibel-Palade bodies and lipid droplets were found. We conclude: The unusual cells may be derived from endothelial cells, from monocytes, or from still immature hematopoetic cells.

S06-11

KNOCK OUT OF MURINE β_3-ADRENOCEPTOR BY HOMOLOGOL RECOMBINATION

Revelli, J.-P., Giacobino, J.-P., *Huarte, J., +Ody, C.
Départements de Biochimie médicale, *Morphologie et +Pathologie, Faculté de Médecine, Centre Médical Universitaire, 1 rue Michel Servet, 1211 Genève 4

In rodents, the β_3-adrenoceptor is expressed mostly in brown and white adipose tissues and has been demonstrated to mediate most of the catecholamine-induced thermogenic and lipolytic effects in these tissues. In order to get more information on the biological role of the β_3-adrenoceptor, a gene targeting technique was used to create mice which lack this receptor. A construct in which, the β_3-adrenoceptor gene was interrupted in its first coding exon by the introduction of a Neomycin resistance sequence, was used to electroporate embryonic stem cells. Two different clones with the desired mutation were obtained and introduced into blastocysts to produce chimeric animals. Viable heterozygote were obtained with both clones. Intercrosses to obtain null allele homozygote offsprings were however unsuccessfull. It is therefore suggested that the knock out of the β_3-adrenoceptor is lethal. In agreement with this, is the unexpected finding that the β_3-adrenoceptor is expressed during early postimplantation stages.

S06-12

IDENTIFICATION OF THE 5-HYDROXYTRYPTAMINE2C RECEPTOR AS A 60 kDa N-GLYCOSYLATED PROTEIN IN CHOROID PLEXUS AND HIPPOCAMPUS

D. Abramowski and M. Staufenbiel
Sandoz Pharma Ltd, Preclinical Research
Basle CH-4002, Switzerland

The rat 5-hydroxytryptamine$_{2C}$ (5-HT$_{2C}$) receptor was identified as N-glycosylated polypeptide of 60 kDa apparent molecular weight using antibodies against cytoplasmic domains. To show that the polypeptides detected on Western blots and by immunoprecipitation represent the 5-HT$_{2C}$ receptor, binding of the 5-HT$_{2C}$ ligand ^{3}H-mesulergine to immunoprecipitates from extracts of pig choroid plexus was performed. We demonstrate the presence of a signal sequence which was cleaved off during membrane insertion resulting in a 38 kDa polypeptide. During further maturation the receptor was N-glycosylated at two sites via a 48 kDa intermediate. This intermediate was far more abundant in choroid plexus than in hippocampus and may represent an intracellular receptor reserve. Transfection of 5-HT$_{2C}$ cDNAs into cultured cells yielded receptor polypeptides that differed from those found *in vivo* due to abnormal N-glycosylation. Thus receptor expressed in cell lines may also differ functionally from the receptor in its native tissue.

S06-13

ROLE OF TNF RECEPTOR I (TNFRI) IN HEMATOPOIESIS

M. Lorenz, V.F.S. Quesniaux‡, B.D. Car*, H. Bluethmann† and B. Ryffel
Swiss Federal Institute of Technology Institute of Toxicology Schorenstrasse 16, CH-8603 Schwerzenbach; ‡ Sandoz Pharma Ltd., CH-4002 Basel, * DuPont Merck, Stine-Haskel-Research Center, P.O. 30, Newark, DE 19714-0030, USA; † Pharmaceutical Research, F. Hoffmann-LaRoche Ltd., CH-4002 Basel

The role of signaling through p55 TNFRI for hematopoiesis was investigated in TNFRI-deficient mice. In steady state no major hematopoietic differences between receptor-positive and negative mice were observed. However, peripheral white leucocyte counts were elevated 30-40% in receptor-deficient mice compared to wildtype, apparently related to increased numbers of lymphocytes. Myelotoxic injury was induced by 5-Fluorouracil (5-FU). The blood cell nadir at day 5 after treatment as well as the usual return to baseline levels at day 15 seen in wildtype mice was also observed for TNFRI-negative mice, but with higher leucocyte levels. Reduced spleen cell numbers at day 5 for both mice strains were followed in 3 out of 4 experiments by strongly elevated numbers in TNFRI-deficient mice. Preliminary data on the recovery after 5-FU in bone marrow of receptor-deficient mice revealed a 2-fold higher number for erythroid progenitors, while the recovery of the granulocyte-macrophage progenitors was enhanced in wildtype mice. Erythropoiesis in the spleen was comparable between both mice strains after 5-FU treatment, while extramedullary granulopoiesis was apparently higher in wildtype mice. Thus, signals mediated through TNFRI seem to play a role not only in steady state hematopoiesis, but also in recovery after myelotoxic insult.

S06-14

THE CONTINUOUS PROLIFERATION OF *THEILERIA PARVA*-INFECTED T-CELLS DOES NOT INVOLVE TCR SIGNALLING.

Galley Y., Hagens G. & Dobbelaere D.
Institute of Animal Pathology, Bern

When T-cells are infected by *T. parva*, they cease to require TCR stimulation and exogenous growth factors to proliferate. Constitutively activated NFκB can be found in the nucleus of infected cells and they also express the IL2 and IL2R genes, events wich under normal circumstances depend on TCR stimulation. We examined the possibility that the parasite interferes with or short-circuits the TCR signalling pathway. In *T. parva*-infected T-cells, the CD3ε and TCRζ chains, which are involved in transducing signals from the TCR to the interior of the T-cell, are not phosphorylated on tyrosine residues, suggesting that the TCR/CD3 complex is not activated. In addition, the proliferation of *T. parva*-infected cells is not inhibited by ascomycin, an immunosuppressive compound related to FK506, which inhibits calcineurin, a key molecule in TCR-mediated T-cell activation and proliferation. These data suggest that *T. parva* by-passes the TCR signalling pathway to induce the uncontrolled proliferation of the T-cells they infect.

S06-15

PLATELET-DERIVED GROWTH FACTOR MODULATES CYTOKINE-INDUCED NERVE GROWTH FACTOR SYNTHESIS IN RAT GLOMERULAR MESANGIAL CELLS

Katharina M.A. Plüss*, Joseph Pfeilschifter†, Heiko Mühl† and Uwe Otten*, *Dept. of Physiology, University of Basel, CH-4051 Basel and †Dept. of Pharmacology Biozentrum, University of Basel, CH-4051 Basel

We have recently shown that inflammatory mediators such as interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) are potent inducers of NGF in peripheral tissues and in brain. Platelet-derived growth factor (PDGF) itself plays a role in tissue repair, embryogenesis, and pathophysiological states marked by excessive cell proliferation such as atherosclerosis and glomerulonephritis. These results indicate that PDGF could be a key mediator of the pathophysiology of inflammatory renal diseases. Using cultured rat glomerular mesangial cells, which play a key role in kidney immune function, we measured NGF mRNA levels by reverse transcription-polymerase chain reaction (RT-PCR), and correlated these to NGF protein levels measured by ELISA. We found that costimulation of rat glomerular mesangial cells with PDGF-BB and IL-1β/TNF-α significantly enhances the IL-1β/TNF-α-induced NGF synthesis. In contrast, preincubation with PDGF-BB drastically reduces NGF gene expression in response to IL-1β/TNF-α stimulation. Our results indicate that PDGF-BB is a potent modulator of cytokine-induced NGF expression in rat mesangial cells, depending on when the PDGF receptor is activated.

S06-16

Ehk-1 a neuronal Elk-like receptor tyrosine kinase in brain: aberrant expression in human glial tumors.

Miescher G.C., Taylor V., Olivieri G., Schröck E.* & Steck, A. J.
Department of Research and Neurological Clinic, 4031 Basel and *Institut für Humangenetik-6900 Heidelberg

Ehk-1 is a novel Elk-related receptor tyrosine kinase detected in all neurones of the central nervous system in the rat[1]. We now report the cDNA cloning of the human homologue and the assignment of the gene to human chromosome 4q12-13. The human Ehk-1 homologue has 95% amino acid identity with the rat sequence. As in the rat there are three main transcripts in the human with sizes of approximately 10, 6.0 and 5.0 kb. There are similar multiple mRNA splice variants as in rat including a novel cDNA without any Fibronectin type III domain in the extracellular part. The diversity at the protein level seems similarly restricted as in the rat with 120 and 115 kDa proteins being produced in normal brain. Surprisingly Ehk-1 expression was also detected as a 120 kDa protein in a lowgrade malignancy astrocytoma and a 110 kDa band in a biopsy of a glioblastoma. By screening of Northern blots 2/15 astrocytomas, 1/17 glioblastomas and 1/17 glioblastoma cell lines expressed *ehk-1*. Immunohistology of one astrocytoma biopsy showed Ehk-1 expression in all tumor cells and adjacent neurons. As its ligand is widely expressed in brain Ehk-1 could contribute to tumor development and phenotype.

[1]Taylor V. et al. 1994: Neuroscience 63(1): 163-176.

S06-17

FUNCTIONAL INTERACTION OF NEUROTROPHIN THROUGH THE LOW AFFINITY RECEPTOR. C. Hertel and A. Müller, Pharma Division, Preclinical Research, F. Hoffmann-La Roche AG, CH-4002 Basel

Functional interaction of the low affinity nerve growth factor receptor LNGFR and the tyrosine kinase receptor TrkA is unresolved. LNGFR is non-selective for neurotrophins, while TrkA is selective for NGF and does not bind BDNF. Functional response to NGF is mediated by TrkA dimerization inducing a phosphorylation cascade. Recent results indicate that LNGFR may act to increase the local concentration of NGF for trkA. To investigate a possible interaction of LNGFR and TrkA, a glial cell expressing LNGFR, but neither TrkA nor TrkB or TrkC, were transfected with TrkA. TrkA transfected 33Bcells, 33B-6, express high and low affinity binding sites for TrkA. Stimulation of 33B-6 cells with NGF results in phosphorylation of TrkA and PLCgamma, and induces formation of cellular processes, while wt-33B cells do not respond. Concomitant treatment with BDNF and NGF reduced binding of NGF not only to LNGFR but also to TrkA, however, phosphorylation of the signalling proteins is enhanced. These results suggest that BDNF alters the signalling of NGF through TrkA by competing for their common receptor LNGFR.

S06-18

Expression of Colony-Stimulating Factor-1 in Murine Osteoblasts

J. Halasy, R. Felix, A. Wetterwald, M. Cecchini, H. Fleisch and W. Hofstetter. Pathophysiology., Uni. Bern, Murtenstrasse 35, 3010 Bern.

Colony-stimulating factor-1 (CSF-1), the growth factor of the mononuclear phagocytic system and for osteoclasts is produced by mesenchymal cells, among them osteoblasts. Transfection experiments with human cDNAs revealed that the cytokine is synthesized as a rapidly secreted or as a membrane bound form. Transcripts containing the complete exon 6, encoding a proteolytic cleavage site that is hydrolyzed, yield a rapidly secreted peptide. Transcripts encoding the membrane bound molecule lack large portions of exon 6, do not encode the cleavage site and the protein remains attached to the membrane spanning domain.

The aim of this study was to investigate by Reverse Transcription Polymerase Chain Raction (RT-PCR) the molecular forms of CSF-1 produced by osteoblastic cells. Primer pairs discriminating the mRNAs containing the altenatively spliced exons 6, 9, or 10 of the CSF-1 gene were chosen. Specificity of the amplicons was verified by Southern hybridization with internal oligonucleotides as well as by sequencing.

In primary cells from murine calvariae and in the osteoblastic cell lines KS-4 and MC3T3-E1, two pairs of CSF-1 transcripts, either with exon 9 or 10 at their respective 3' ends, are present. Of the mRNAs with identical 3' ends, one contains the intact exon 6 and encodes the rapidly secreted form of CSF-1, while the other mRNAs lack large portions of exon 6 and encode the membrane associated form of the cytokine.

In conclusion, osteoblastic cells contain transcripts encoding both secreted and membrane integrated CSF-1. Since in bone osteoblasts and osteoclastic precursors and mature osteoclasts are in close proximity, the locally produced and presented form of the cytokine may play a decisive role in formation and/or activation of osteoclasts.

S06-19

Expression of p75^{NFGR} in a human neuroblastoma cell line alters signalling mediated by TrkA

C. Hertel and P. Kuner, Pharma Division, Preclinical Research, F. Hoffmann-La Roche Ltd, CH-4002 Basel

Nerve growth factor (NGF) binds to two distinct receptors, the high affinity NGFR TrkA, a tyrosine receptor kinase and the low affinity p75^{NFGR} whose role in signal transduction is still unclear. A human neuroblastoma cell line, expressing only TrkA was transfected with p75^{NFGR} to investigate the functional interaction of p75^{NFGR} and TrkA in signal transduction. Transfected IMR-32 clones are demonstrated to express p75^{NFGR} mRNA, which was enhanced by stimulation by dibutyrylcAMP and to a lesser extent by rhNGF and phorbol ester (TPA). The effect of p75^{NFGR} on TrkA dependent signal transduction, as determined by tyrosine phosphorylation of TrkA and downstream signaling proteins was investigated.

S06-20

B16 melanoma cells selected *in vivo* for enhanced liver metastasis express high amount of HGFr (c-met protooncogene)

D.Rusciano, P.Lorenzoni and M.M. Burger, Friedrich Miescher Institut, P.O. Box 2543, CH-4002 Basel

Hepatocyte Growth Fator (HGF) is a potent mitogen for hepatocytes and has been found to be identical to Scatter Factor (SF), a motility factor secreted by mesenchymal cells and active on epithelial cells. The cellular receptor for HGF/SF is the product of the c-met proto-oncogene, a tyrosine-kinase receptor which mediates all the activities triggered by the factor. Here we report that B16-LS9 cells (selected *in vivo* for liver-specific metastasis) have a higher tyrosine kinase activity than their parental B16-F1 cells, and that a high expression of c-met is mostly responsible for such increase. Moreover, in other independent selections with B16 cells we also found that liver colonies always expressed higher amounts of c-met, suggesting that high c-met expression is a requirement for liver colonization by B16 melanoma cells. However, c-met activation in B16 cells appears to have negative effects on cell growth, while it highly stimulates cell motility, suggesting that its role in liver colonization could be related to the invasion step.

S06-21

INFLUENCE OF GROWTH FACTORS ON REMODELING OF CYTOSKELETAL AND CONTRACTILE STRUCTURES IN ADULT RAT CARDIOMYOCYTES IN CULTURE

Harder, B.A., Eppenberger-Eberhardt, M.* and Schaub, M.C.
Institute of Pharmacology, University of Zürich, CH-8057 Zürich; and
*Institute of Cell Biology, ETH-Zürich, CH-8093 Zürich, Switzerland

The effects of the growth factors bFGF and IGF-I on structural (actin cytoskeleton and myofibrillar apparatus) remodeling and on the functional aspect of atrial natriuretic factor (ANF) expression in adult rat ventricular cardiomyocytes have been followed during the hypertrophy reaction in long-term cell cultures. Cells attach to the substratum and spread into polygonal shapes with pseudopodia. A well structured actin network fills the cell bodies and the extensions. Untreated cells and cells with IGF-I double in volume while bFGF induces a four-fold increase. The myofibrillar apparatus follows the actin stress fiber-like structures in growing out into the cell periphery. Immunoreactive ANF granules develop around the nuclear region. The fetally occurring α-smooth muscle actin (α-sm-actin) is transiently re-expressed in stress fiber-like structures. IGF-I down-regulates α-sm-actin and ANF and promotes myofibrillar growth. bFGF has the opposite effect by up-regulating α-sm-actin and ANF. In addition, bFGF restricts myofibrillar growth with a sharp boundary in the nuclear region. α-sm-actin accumulates in stress fiber-like structures emanating from the myofibrillar area. This seems to imply the notion that α-sm-actin inhibits the outgrow of sarcomeres.

S06-22

DNA BINDING OF THE ECDYSTEROID RECEPTOR-ULTRASPIRACLE HETERODIMER TO NATURAL AND SYNTHETIC ECDYSTEROID-RESPONSE ELEMENTS

Carsten Elke, Martin Vögtli and Markus Lezzi
Institut für Zellbiologie, ETH Hönggerberg, CH-8093 Zürich

The specificity of two nuclear receptors from insect, ecdysteroid receptor (EcR) and Ultraspiracle (USP), was compared with respect to their DNA binding and hetero-/homodimerization properties by means of electrophoretic mobility shift assays (EMSA). The two receptors from the insect species, *Chironomus tentans* and *Drosophila melanogaster*, respectively, were either expressed as GST-fusion proteins in *E.coli* or generated by an *in vitro* translation reaction.

It had been demonstrated previously that the *Drosophila* EcR requires USP as a partner in a heterodimer complex for high affinity binding to an ecdysteroid response element (EcRE) of *hsp27*. We could confirm this finding with protein generated by *in vitro* translation or in *E. coli*. However high affinity binding of GST-EcR (generated in *E. coli*) to a synthetic EcRE, composed of two AGGTCA half-sites in a palindromic array with a 1 bp spacing (PAL-1), was achieved also in the absence of USP. The position of the retarded protein-DNA complex in EMSA indicated that the PAL-1 is recognized by an EcR homodimer. In contrast, *in vitro* translated EcR showed a very low affinity to PAL-1 and no binding to the *hsp27*-EcRE in the absence of USP. PAL-1, like *hsp27*-EcRE, constituted a high affinity binding site for the EcR/USP heterodimer. A 2 to 3-fold enhancement of DNA binding was observed when hormone at a concentration of 10^{-6} M was added to the binding reaction.

S06-23

Characterization of a novel motif for protein kinase C phosphorylation in the Na,K-ATPase.

P. Beguin, A. T. Beggah, M.C. Peitsch* and K. Geering
Institut de Pharmacologie and Toxocologie de l'Université, 1005-Lausanne and *Glaxo Institute of Molecular Biology, 1228-Plan-les Ouates

The α subunit (Sα) of Na,K-ATPase is phosphorylated by protein kinase C (PKC) *in vitro* and in intact cells (Beguin et al., JBC 269, 24437, 1994). The mayor PKC phosphorylation sites in the N-terminus of Sα at Ser15 is located in a novel PKC motif (^{7}RDKYEPAA^{15}TS^{16}EHGGKK^{22}). Based on a computer modelling of the interaction between bovine PKC and the N-terminus of Sα, we focused on the importance of the basic amino acid residues surrounding Ser16 to characterize the structural requirements for PKC phosphorylation. Mutants were prepared, expressed in Xenopus oocytes and their phosphorylation capacity tested in homogenates upon PMA stimulation. Replacement of the positively charged His18 with Ala or Asp abolished while replacement with Arg preserved PKC phosphorylation. Asn and Gln reduced phosphorylation by about 50%. Phosphorylation was not restored in the Ala18 mutant by introducing a lysine residue at position 19. Finally, replacement of Arg7 and Lys9 or Lys22 and Lys23 by Ala led to an about 50% decrease in phosphorylation. In conclusion, the results demonstrate that efficient phosphorylation of Ser16 depends on 1) the hydrogen bonding capacity and the position at +2 of His18 and 2) the presence of several other positively charged residues located more distantly at -7 to -9 and +5 to +6.

S06-24

ACTIVATION OF PROTEIN KINASE C IN CARDIOMYOCYTES INDUCES PHOSPHORYLATION OF PHOSPHATASE 2A AND MICROTUBULE ASSOCIATED PROTEIN KINASE.

Braconi Quintaje S., Church D.J., Vallotton M.B. and Lang U. Division of Endocrinology, University Hospital, CH-1211 Geneva 14.

The microtubule associated protein kinase (MAP K) and the phosphatase 2A (PP2A) are two important enzymes, implicated in the cellular signal transduction. The influence of protein kinase C (PKC) activation on PP2A and on MAP K was studied in spontaneously beating ventricular cardiomyocytes from neonatal rats. PKC-induced phosphorylation was determined by using the combined techniques of immunoprecipitation and electrophoresis.

The PKC activator 12-phorbol 13-myristate acetate (PMA) induced the phosphorylation of both enzymes within 5 minutes. It also induced a transient increase in the activity of MAP K reaching a maximal stimulation of 210 ± 30 % of control values at 5 minutes. In contrast, PP2A activity was abolished after 5 min of exposure to PMA but this inhibition was reversed after 15 min. Thus, PKC-induced phosphorylation regulates PP2A and MAP K activity in an opposite way. Our results indicate that reversible protein phosphorylation is crucial for the control of enzyme activities involved in cell signaling.

S06-25

CHARACTERIZATION OF RAT MESANGIAL CELLS EXPRESSING PROTEIN KINASE C ISOENZYME-SPECIFIC ANTISENSE cDNA'S

Richard Hummel and Josef Pfeilschifter, Dept. Pharmacology, Biozentrum, University Basel, Klingelbergstr. 70, CH-4056 Basel, Switzerland

Glomerular mesangial cells are involved in the regulation of the renal filtration rate and take part in inflammatory processes of the glomerulus. Protein kinase C (PKC), a serine-threonine kinase with at least 11 different known isoforms is thought to play a key role in triggering some of these processes. Mesangial cells express the PKC isoforms α, δ, ε and ζ. To investigate the role of distinct isoenzymes, we made stable transfections of rat mesangial cells with antisense cDNA's to the corresponding PKC isoforms. Established cell lines were characterized with respect to content of PKC isoenzymes, growth and proliferation, the ability of the remaining isoforms to influence physiological functions of these cells.

S06-26

IDENTIFICATION OF PROTEINS THAT BIND TO THE REGULATORY DOMAIN OF PROTEIN KINASE Cγ

Quest, A.F.G., Rousseau, M., Hunn, M., and Bron, C.
Institute of Biochemistry, University of Lausanne

Cysteine-rich motifs (Cys1, Cys2) within the Protein kinase C (PKC) regulatory domain mediate lipid interactions critical for phorbol ester- and diacylglycerol-dependent regulation of PKC activity. Additional lipid interaction sites lie within the first variable (V1) and second constant (C2) region of calcium-dependent PKCs. We tested the hypothesis that lipid-binding elements of PKC may have additional roles and, for instance, mediate protein-protein interactions. Regulatory domain elements of a calcium-dependent isoform, PKCγ, were expressed in E. coli as glutathione-S-transferase (GST) fusion proteins, purified by affinity chromatography and utilized in a ligand-blot assay to identify PKC binding proteins in cell extracts after transfer to nitrocellulose. Several proteins of molecular mass 14-18 and 28-33 kDa were found to bind to the regulatory domain of PKCγ in the absence of lipids. Binding was observed with a GST-Cys2C2 fusion protein, but not with GST-V1Cys1. Both GST-Cys2 and GST-C2 bind independently to the same proteins, which are present in Triton X100 extracts of various tissues (brain, lung, kidney, liver, heart, testes, ovaries). Such widespread, lipid-independent PKC-protein interactions detected in vitro may contribute significantly to PKC function in vivo.

S06-27

ACTIVATION OF PROTEIN KINASE C IN CARDIOMYOCYTES INDUCES PHOSPHORYLATION OF PHOSPHATASE 2A AND MICROTUBULE ASSOCIATED PROTEIN KINASE.

Braconi Quintaje S., Church D.J., Vallotton M.B. and Lang U. Division of Endocrinology, University Hospital, CH-1211 Geneva 14.

The microtubule associated protein kinase (MAP K) and the phosphatase 2A (PP2A) are two important enzymes, implicated in the cellular signal transduction. The influence of protein kinase C (PKC) activation on PP2A and on MAP K was studied in spontaneously beating ventricular cardiomyocytes from neonatal rats. PKC-induced phosphorylation was determined by using the combined techniques of immunoprecipitation and electrophoresis.
The PKC activator 12-phorbol 13-myristate acetate (PMA) induced the phosphorylation of both enzymes within 5 minutes. It also induced a transient increase in the activity of MAP K reaching a maximal stimulation of 210 ± 30 % of control values at 5 minutes. In contrast, PP2A activity was abolished after 5 min of exposure to PMA but this inhibition was reversed after 15 min. Thus, PKC-induced phosphorylation regulates PP2A and MAP K activity in an opposite way. Our results indicate that reversible protein phosphorylation is crucial for the control of enzyme activities involved in cell signaling.

S06-28

CONFORMATION AT THE CASEIN KINASE II SITE NEAR THE NUCLEAR LOCALISATION SIGNAL OF SV40 LARGE T-ANTIGEN AFFECTS NUCLEAR TRANSPORT

David A. Jans, Chong-Yun Xiao, Stefan Huebner, Lyndall J. Briggs and Patricia Jans; Division for Biochem. & Mol. Biol., John Curtin School of Medical Research, A.N.U., Canberra, Australia

Nuclear import of SV40 large T-antigen fusion proteins is greatly enhanced by the casein kinase II (CKII) site (SSDDE$^{111-115}$) flanking the nuclear localisation sequence (NLS) (PKKKRKV^{132}). Aspartic acid at either position 111 or 112 can mimic phosphorylation at the site, thereby enhancing nuclear transport. To examine the importance of the spatial arrangement of the CKII site and NLS, we used site-directed mutagenesis to place the proline at position 110 one residue either upstream or downstream relative to Asp^{112} and analysed the effects on nuclear import at the single cell level both in vivo and in vitro using quantitative fluorescent techniques. Proline at position 109 or 111 reduced transport by 50-75% compared to the Pro^{110}Asp^{112} configuration. In preliminary experiments to test the importance of spacing between the CKII site and NLS, we reduced the spacer from 10 to 4 amino acids. This markedly reduced nuclear import to 25-30 % wild type, although not affecting CKII phosphorylation. The results imply that both protein conformation at the CKII site and spacing between it and the NLS may be crucial for correct recognition during the nuclear transport process. The CKII site and NLS may conceivably be recognised in concert.

S06-29

LOCALIZATION OF POLYOMAVIRUS MIDDLE-TUMORANTIGEN IN RAT EMBRYO FIBROBLASTS

Messerschmitt, A., Ballmer-Hofer, K., FMI,Basel

Polyomavirus middle-T and large-T antigen have been shown to be both necessary and sufficient for the establishment of transformed cells in culture. Middle-T acts like a constitutively activated growth factor receptor initiating a cascade of signal-transducing pathways. We are interested in the relationship between the localization of middle-T in a cell and its function. Middle-T carries a carboxy-terminal membrane anchor and is localized in a perinuclear membrane compartment, probably representing the endoplasmatic reticulum (ER). This result may imply that middle-T is not targeted to the plasma membrane as shown earlier and that it interacts with components of cellular signaling pathways in the ER. We removed the membrane anchor of middle-T and found that the protein was now present in the nucleus. Mutations in the amino-terminus of this truncated middle-T abrogated the nuclear targeting resulting in cytoplasmic localization. Since the amino-terminal mutations are known to block the association of middle-T with cellular proteins we suggest that middle-T lacking the membrane anchor is targeted to the nucleus through binding to cellular proteins, as e. g. phosphatase 2A.

S06-30

THE ROLE OF POLYOMAVIRUS MIDDLE-ANTIGEN IN CELLULAR SIGNALING

Marc Urich, Mahmoud El-Shemerly and Kurt Ballmer-Hofer; FMI Basel

Polyomavirus middle-T antigen, one of the early gene products of the virus, transforms cells in culture.It associates with various cellular proteins, namely the protein tyrosine kinases c-Src, c-Fyn, Yes, phosphatase 2A, phosphatidylinositol 3-kinase (PI3-K), the adaptor protein Shc and a recently identified member of the 14.3.3 family. In analogy to recent findings for growth factor receptors it is tempting to speculate that the binding of Shc to middle-T leads to activation of the MAP-kinase pathway. We could demonstrate that MAP-kinase is constitutively activated in middle-T transformed NIH 3T3 cells. Furthermore we found that a middle-T mutant lacking the PI3-K binding site is not able to activate MAP-kinase in 3T3 cells. This suggests that a crosstalk between the PI3-K and Map-kinase pathways is required for middle-T induced transformation. In future experiments we will concentrate on the identification of the crosspoint(s) between these two pathways.

S06-31

Cytosolic localization of the eph-related receptor PTK myk-1.

Daniel Albrecht, Gisela Zürcher, Andrew Ziemiecki and Anne-Catherine Andres
AKEF, Department of Clinical Research, Tiefenaustrasse 120, CH-3004 Bern

We have recently isolated two novel members of the eph-related family of receptor protein tyrosine kinases (PTKs) from mammary gland tissue, myk-1 and myk-2, whose expression suggests their involvement in the control of epithelial proliferation. Myk-2 specific antibodies recognized a glycosylated protein of 130 kd with associated kinase activity. Myk-1 specific antibodies, raised against either the N-terminal or the C-terminal part of the protein, recognize a 100 kd protein in Western blots but failed to recognize the protein in immunoprecipitation (IP) of cell lysates. In vitro transcription/translation of myk-1 full-length cDNA gave rise to a 100 kd protein which was detected both by Western blotting and IP. The addition of dog pancreas membranes during the in vitro translation had no effect on the myk-1 protein. Subcellular fraction of cell lysates showed that myk-1 was mainly localized in the soluble fraction, whereas the myk-2 protein was associated with the membrane fractions. From these data we conclude that myk-1, although from the nucleic acid sequence showing all the characteristic features of a receptor PTK, fails to insert into the rough ER membranes during synthesis, is not glycosylated and is not membrane associated. We are currently examining the implications of this unique behavior of a receptor PTK for its function as a putative signal transducing molecule.

S06-32

The *Syk* protein tyrosine kinase is expressed in mammary epithelial cells.

Martin Flück, Gisela Zürcher, Anne-Catherine Andres and Andrew Ziemiecki
AKEF, Department of Clinical Research, Tiefenaustrasse 120, CH-3004 Bern

We have used a PCR based cloning strategy to identify protein tyrosine kinases (PTKs) involved in mammary gland biology of the mouse. This approach led to the identification of a cDNA fragment showing high homology to the intracellular PTK *syk*. We have isolated the corresponding full-length cDNA. Sequence analysis revealed the presence of two SH2 domains and a catalytic domain and comparison with the previously published sequences of human, rat and porcine *syk* confirmed its identification as murine *syk*. Northern blot analysis showed that *syk* is expressed as two transcripts of 4 and 5 kb in size with highest levels in spleen, thymus and mammary gland. In the mammary gland *syk* is expressed in a differentiation specific manner being induced around puberty, peaking during the estrus cycle of mature females and being repressed during the pregnancy induced differentiation. *Syk* has been described as being specific for cells of haematopoietic origin. Indeed, a major part of *syk* expression observed in the mammary gland could be assigned to the lymph nodes, however, expression was also detected in the epithelial compartment and in established mammary epithelial cell lines of mouse and human origin. This suggests that *syk* may not only be involved in B- and T-cell signaling but may function in other (epithelial?) cellular pathways. We have raised antibodies specifically recognizing the 70 kd *syk* protein. With the help of this antiserum we intend to identify cell types expressing the *syk* gene in the mammary gland and to elucidate the role of *syk* expression in the homeostasis of the mammary gland.

S06-33

ASSOCIATION OF POLYOMAVIRUS MIDDLE-T ANTIGEN WITH SRC FAMILY KINASES

Dunant, N., Senften, M., and Ballmer-Hofer, K.
Friedrich Miescher-Institut, Basel

Polyomavirus tumor antigens (T antigens) are responsible for tumorigenesis of this virus in animals and for viral transformation of cells in culture. One of the T antigens, middle-T antigen, is known to associate with and thereby activate cellular tyrosine kinases like c-Src, Fyn, c-Yes, whereas no association with other highly related kinases such as Hck and Lck has been observed. We have shown that neither myristylation nor the SH3 domain of c-Src are required for association with middle-T. In order to map the site(s) on c-Src responsible for association with middle-T we constructed hybrid kinases consisting of sequences derived from c-Src and Hck and tested their ability to bind middle-T. Identification of the c-Src sequences interacting with middle-T is aimed at the isolation and characterization of cellular proteins that show functional homology with middle-T.

S06-34

Protein-protein interactions mediated by the JH domains of JAK1 protein

Peter Küng, Gisela Zürcher, Anne-Catherine Andres and Andrew Ziemiecki
AKEF, Department of Clinical Research, Tiefenaustrasse 120, CH-3004 Bern

Protein tyrosine kinases (PTKs) are integral components of the cellular machinery that mediates the transduction and processing of many extra- and intracellular signals. Members of the JAK family of intracellular PTKs are characterized by the possession of a PTK related domain N-terminal to the bona fide kinase domain and five additional domains conserved to a greater or lesser extent between the different members and referred to as *JAK homology* or JH domains. An important breakthrough in the understanding of JAK kinase function(s) has come from the recent observations that many cytokine receptors compensate their lack of a PTK domain by utilizing members of the JAK family for signal transduction. JAK1 has been shown to participate in the signal transduction initiated by prolactin, growth hormone and colony stimulating factor-1, three cytokines that have been implicated in growth and differentiation of the mammary gland. JAK1, in contrast to the other members of this family, contains a putative nuclear localization signal and part of the protein localizes to the nucleoli. The JAK1 protein can be readily isolated in complexed form from mammary derived cell lines. We have set out to investigate the involvement of the JH domains of JAK1 in protein-protein interactions occurring during the JAK mediated signal transduction from the cell surface to the nucleus in mammary tissue. With the help of isolated JH domains and the yeast two hybrid system we have begun defining protein-protein interactions with a view to understanding signal transduction involving the JAK1 protein.

S06-35

PtdIns(3,4,5)P3 DOES NOT CONTROL GELSOLIN-ACTIN DISSOCIATION IN HUMAN NEUTROPHILS

Arcaro, A and Wymann, M.P. Institute of Biochemistry, University of Fribourg, CH-1700 Fribourg

Stimulation of neutrophils with chemoattractants induces the dissociation of gelsolin from the barbed ends of actin filaments in human neutrophils, an event that has been proposed to initiate actin polymerization.
We have studied the effects of polyphosphoinositides on gelsolin-actin complexation in vitro and used wortmannin, a selective inhibitor of PtdIns 3-kinase to investigate the importance of PtdIns(3,4,5)P_3 in the regulation of the levels of complexes in vivo.In vitro, PtdIns(3,4,5)P_3 was found to be equally potent to PtdIns(4,5)P_2 in dissociating gelsolin-actin complexes (EC_{50}=19 μM) and slightly more potent in inhibiting the severing activity of gelsolin (EC_{50}=4 μM vs. 10 μM). Considering the low concentrations of PtdIns(3,4,5)P_3 in respect to PtdIns(4,5)P_2 within cells, these results suggest that PtdIns(3,4,5)P_3 may not be an effective regulator of gelsolin-actin complexation. Moreover, pretreatment of neutrophils with wortmannin did not affect the fMLP-induced decrease in EGTA-resistant gelsolin-actin 1:1 complexes and affected the rise in barbed-end nucleating activity but only at micromolar concentrations. Ca^{++}-depletion strongly lowered basal levels of EGTA-resistant gelsolin-actin 1:1 complexes, probably by affecting the reassocition of the two proteins.This implies that gelsolin-actin dissociation is not sufficient to induce actin polymerization, since this cell response is still functional in the absence of calcium.

S06-36

SIGNAL TRANSDUCTION IN *THEILERIA PARVA*-INFECTED T-CELLS.

Hagens G., Eichhorn M., Galley Y., *Boscoboinik D. & Dobbelaere D., Inst. of Animal Pathology and *Inst. of Biochemistry and Molecular Biology,Bern

T-cells that are infected by the parasite *T. parva* acquire the ability to proliferate continuously. We are examing the different signal transduction pathways with which the parasite could interfere to induce continuous T-cell proliferation. The serine/threonine kinase Raf, which plays a crucial role in T-cell activation is hyperphosphorylated and is membrane-associated in *T. parva*-infected cells. The hyperphosphorylated form disappears when the parasite is eliminated from the host cell, however, and membrane translocation is strongly reduced. We also studied PKC, which has been shown to be a potent activator of the Raf-MAP kinases pathway in T-cells. In infected cells, the stimulation of PKC results in only a partial activation of MAP kinases. Interestingly, and in stark contrast to uninfected mitogen-stimulated T-cells, the PKC inhibitor bisindolylmaleimide has no effect on the proliferation of *T. parva*-infected T-cells. PKC activation, translocation and susceptibility to downregulation by phorbol esters are presently being examined.

S06-37

INTRACELLULAR EXPRESSION OF A SINGLE CHAIN DERIVATIVE OF A c-Raf SPECIFIC MONOCLONAL ANTIBODY.

E. Stöcklin, M. Wissler, W.Wels and B. Groner, Institute for Experimental Cancer Research, Tumor Biology Center, Freiburg, Germany.

The c-Raf serine/threonine protein kinase plays a central role in mitogenic signal transduction pathways. It is activated by mitogenic agents like growth factors. It links the receptor tyrosine kinases and c-ras to the cytoplasmic protein kinase cascade. Interference with the function of the c-raf-1 kinase could yield insights into the signal transduction pathways and could possibly serve as a cytostatic strategy. We have used the intracellular expression of a single chain antibody (scFv) directed against c-Raf-1 to achieve this aim. The scFvs were derived from the mRNA of hybridoma cells expressing the monoclonal antibodies PBBJ and URP by a combination of RT and PCR. They are composed of the heavy and light chain variable domains connected by a flexible peptide linker. The purified scFvs bind to the raf protein in vitro. The scFv cDNA was introduced into NIH3T3 cells under the control of the inducible MMTV-LTR. The influence of scFv expression on the c-Raf-1 kinase activity, MEK, MAPK phosphorylation and activity and c-fos gene induction were measured.

S06-38

CROSS-TALK BETWEEN SECRETORY PLA_2 AND CYTOSOLIC PLA_2 IS MEDIATED BY MAP KINASE

Andrea Huwiler, Georgia Staudt, Ruth Kramer* and Josef Pfeilschifter, Dept. Pharmacology, Biozentrum, Univ. Basel, Switzerland; *Lilly Research Laboratories, Indianapolis, Indiana, USA

Incubation of rat mesangial cells with proinflammatory cytokines like interleukin 1β triggers the expression of a secretory group II phospholipase A_2 ($sPLA_2$) in rat mesangial cells and increases the formation of prostaglandin E_2 (PGE_2). We show here that $sPLA_2$ acts in an autocrine fashion on the mesangial cells and induces a rapid activation of protein kinase C (PKC) isoenzymes-δ and -ε and of mitogen-activated protein kinase (MAPK). Subsequently a phosphorylation and activation of high molecular weight cytosolic PLA_2 ($cPLA_2$) is observed. The effect of $sPLA_2$ is mimicked by lysophosphatidylcholine (LPC), a reaction product of $sPLA_2$. LPC stimulates MAPK phosphorylation and $cPLA_2$ activation with a subsequent increase in arachidonic acid release. In addition LPC also activates PKC-ε isoenzyme. These data suggest that $sPLA_2$ by cleaving membrane phospholipids and possibly by generating LPC activates $cPLA_2$ via the PKC/MAPK signalling pathway. Obviously a network of interactions between different PLA_2's is operative in mesangial cells and may contribute to the progression of glomerular inflammatory processes.

S06-39

PROMOTER ANALYSIS OF A GROWTH FACTOR REGULATED GENE.

Kessler, R., Laursen, N.B, Trüb, T., Kalousek, M.B., and Klemenz, R. Department of Pathology, Division of Cancer Research, University Hospital, CH-8091 Zürich, Switzerland.

Stimulation of quiescent cells to enter the cell cycle results in altered gene expression. Some of the first genes to be induced (immediate early (i.e.) genes) encode transcription factors which are thought to pass on the mitotic signal to the delayed early (d.e.) genes.

We have analysed the promoter elements required for growth factor mediated induction of the d.e. mouse gene T1 which encodes a secreted glycoprotein of the immunoglobulin superfamily. A 448 bp region located between 3.6 and 4.0 kb upstream of the transcription start site is sufficient for growth factor mediated inducibility. It contains an AP-1 binding site which is absolutely required for T1 gene expression. The AP-1 binding site is surrounded by three E-boxes of which at least two are essential for efficient gene induction. Thus members of the i.e. proteins encoded by the *fos* and *jun* gene family which form the AP-1 complex can activate the d.e. gene T1 in collaboration with basic helix-loop-helix (bHLH) transcription factors binding to the E-boxes. Preliminary results suggest that in addition to the AP1 complex one of the bHLH proteins is also induced /upregulated by serum stimulation.

We are currently analysing the specific influence of various bHLH proteins and different Fos/Jun complexes on the transcription efficiency of the T1 gene. A possible cooperation between the bHLH proteins and the AP-1 complex is also being investigated.

S06-40

IDENTIFICATION OF PROTEINS BINDING SPECIFICALLY TO GM-CSF mRNA 3'UTR SEQUENCES.

Verena Bichsel, Alfred Walz und Matthias Bickel. Laboratory of Oral Cell Biology und Theodor Kocher Institute, University of Bern

The 3'UTR of murine GM-CSF mRNA contributes strongly to the posttranscriptional regulation of the expression of this hemopoietic growth factor. Degradation of GM-CSF mRNA is mediated by AU-rich sequence elements (ARE) and specific binding proteins. Stabilization of the mRNA in T-cells after treatment with phorbol ester (TPA) has been demonstrated to require sequences up-stream of the ARE. By using these specific sequences from the 3'UTR of GM-CSF as probes we could reveal specific binding to cytosolic protein extracts from either murine (EL-4) or human T-cells (Jurkat). By using a novel membrane-based binding assay we detected differential binding when probes were used that included AREs or the upstream regulatory sequences respectively. Specificity of the interaction was shown by competition with probes lacking the appropriate target or with heterologous sequences. Furthermore, we identified strong binding of radiolabelled RNA to a 101 and a 88 kDA protein by Northwestern analyses. This interaction was competed with specific but not with nonspecific, yet polyadenylated RNA. Supported by grant: SNF 31 365 06.92

S06-41

***IN VIVO* MAPPING OF THE *CIS*-ACTING ELEMENTS RESPONSIBLE FOR THE IL2 INDUCIBILITY OF THE IL2 RECEPTOR α GENE**

Rusterholz, C., Pla, M., Mirkovitch, J., Nabholz, M., ISREC, 1066 Epalinges

IL2 receptor α chain (IL2Rα) expression at the cell surface of T cells is induced by concanavalin A and IL2 and leads to the formation of an active IL2 receptor complex. This induction occurs at the transcriptional level. Previous work from our laboratory has shown that a 2.7 kb fragment of the IL2Rα gene 5' flanking region is sufficient to confer this tissue specific inducibility in transgenic mice. *Cis*-acting elements required for IL2 responsiveness, IL2rE, have been mapped to a 78 bp segment at -1.3 kb by transient transfection studies. *In vitro* experiments detect several proteins binding to IL2rE, whose binding activity is not dependent on IL2. However, IL2Rα expression correlates with the appearance of a DNase I hypersensitive site at -1.3 kb, suggesting inducible DNA-protein interactions. Therefore, we are conducting *in vivo* footprinting experiments to identify at the nucleotide level sequences that stably bind factors during the stimulated transcription.

S06-42

CHARACTERIZATION OF VIP- AND NORADRENALINE-INDUCED PROTEINS AND mRNA IN MOUSE ASTROCYTES BY 2-D GEL ELECTROPHORESIS (2D-PAGE) AND BY mRNA DIFFERENTIAL DISPLAY (DD).

G. Pellegri, J-R. Cardinaux and P.J. Magistretti. Institut de Physiologie, Université de Lausanne, Lausanne, Switzerland.

In primary cultures of mouse cerebral cortical astrocytes, a rapid glycogenolysis followed by a massive glycogen resynthesis (six- to ten-fold over basal levels after 9 hr) are induced by vasoactive intestinal peptide (VIP) or noradrenaline (NA). Both actions of the neurotransmitters are mediated by cAMP. Since the induction of glycogen resynthesis triggered by VIP or NA is abolished by inhibition of transcription and translation, we applied the 2-D PAGE and the mRNA DD techniques to search for newly synthetized astrocytic gene products induced by VIP or NA. The comparison of ^{35}S-labeled proteins from primary astrocyte cultures treated or not with VIP 1 μM or NA 100 μM reveals at least 7 proteins in which labeling is increased after the treatment. We focused our attention on the proteins which seemed abundant enough for purification and microsequencing. As a complementary approach, the identification of VIP- and NA-modulated genes has been carried out by the technique of DD. Using this technique we have observed several differences between the patterns of amplified mRNAs from untreated cultures and from cultures exposed to VIP 1 μM or NA 100 μM. cDNA fragments differentially expressed in astrocytes can be isolated from the gel and cloned into vectors. We are presently verifying by Northern blot that astrocytes treated with VIP or NA express higher levels of the mRNAs identified by DD.

S06-43

REGULATION OF TRANSCRIPTION FACTOR CTF/NF-I BY TRANSFORMING GROWTH FACTOR β.

A. Alevizopoulos, T. von der Weid and N. Mermod
Institut de Biologie animale, Uni Lausanne, 1015 Lausanne

CTF/NF-I proteins are sequence specific DNA-binding transcriptional activators implicated in the regulation of several cellular and viral genes. One member of this family, CTF-1, possesses a proline-rich transcription activation domain. We have analyzed the regulation of CTF-1 transcriptional activity by Transforming Growth Factor β (TGFβ) in mouse NIH3T3 cells. We have demonstrated that TGFβ specifically induces CTF-1 transcriptional activity. We have further identified the responsible determinants of hormone effects on a 20aa-peptide at the C-terminus of CTF-1 proline-rich domain. Moreover, we have generated mutations in this sequence that interfere with growth factor regulation. These and other experimental data will be discussed regarding mechanisms for the regulation of CTF/NF-I transcriptional activity.

S06-44

NEGATIVE GENE REGULATION BY A POLY-ALA CONTAINING GLUCOCORTICOID RECEPTOR MUTANT

Martin Hug; Tiziano Tallone; Sandro Rusconi; *Institut für Molekularbiologie II der Universität, UZ Irchel, Winterthurerstrasse 190, 8057 Zürich, Switzerland; Institut de Biochimie, Perolles, Ch1700 Fribourg*

We have recently obtained a mutant glucocorticoid receptor that is unable to activate a reporter gene driven by the MMTV promoter (Lanz & *al.*, Nucl. Acids Res, in press). The mutant had been obtained by altering the local reading frame of a CAG repeat that normally encodes for a poly-Gln stretch contained in the rat GR cDNA. The alteration that desactivates the GR forces the translation of the CAG into a poly-Ala stretch. The GR[Ala] mutant is able to competitively inhibit wild type GR. In order to better understand the mechanism of desactivation, we have compared the effects of poly-Ala stretches of different length when linked to the GR or to chimeric GAL4 factors with the effects of another simple "repressor" sequence consisting of (Lys)4-Ala repeats (Saha & al. *Nature* **363**, 648-652). We found that the Lys-Ala motif does not desactivate the GR and that the mechanism of transcription desactivation by the poly-Ala and the Lys-Ala motifs is very different. More interestingly, we found distinct effects when the GR[ala] is asked to act as a promoter or as an enhancer activating molecule. We are currently analyzing the effect of poly-Ala motifs on the ability of the GR to act as a natural repressor of oncogene-dependent promoters (Yoshinaga & al., *Mol Endocrinol* **5**, 844-53 and refs, therein)

S06-45

Signal transduction in human muscle after a single bout of exercise

H. Heider, A. Puntschart, H.-R. Widmer, K. Jostarndt, H. Hoppeler, R. Billeter
University of Bern, Department of Anatomy, Bühlstr. 26, 3012 Bern, Switzerland

The initial molecular events in human muscle that lead to the well known morphological adaptations of human muscles to altered demands are not well understood. In order to shed some light on this chain of events, we investigated whether classical intracellular signal transduction pathways are acting also in stressed muscles.

Volontary subjects were running on a treadmill for half an hour at a speed equivalent to their anaerobic thresholds. Biopsies from *M. vastus lateralis* were taken before and at several time points after the run. Cryosections from these biopsies were homogenized and either processed for in-gel kinase assays, western blots or immunoprecipitations.

In-gel kinase assays did not reveal any MAP kinase activity, though the kinase was expressed as shown by western blotting. Moreover, it was activatable in vitro, since incubation of small pieces of muscle with EGF, a well known activator of MAP kinases, induced MAP kinase phosphorylation and subsequent activation as revealed by the retarded electrophoretic mobility of the correponding bands and positive in-gel kinase assays. Preliminary quantitative PCR data suggest that CL100, a recently characterized dual specificity phosphatase that dephosphorylates and thereby inactivates MAP kinases, might be responsible for suppressing MAP kinase activity in stressed muscle, since the message of this phosphatase is upregulated after a bout of exercise.

Immunoprecipitated SAPK activity was assayed using a fusion protein containing the N-terminus of c-jun. Around 10fold increase of SAPK activity was detected in the biopsies taken 6 and 30 minutes after the run. At 3h after the run, activity was lower, close to the value observed before exercise. SAPK phosphorylates c-jun in its transactivation domain (Pulverer et al., Nature 353 (1991), pp. 670-673) and could therefore initiate increased expression of genes containg AP1 sites in their promoter region, including c-jun itself.

S06-46

THE ROLE OF DIFFERENT JUN MEMBERS ON MAMMARY EPITHELIAL CELL DIFFERENTIATION *in vitro*

G. Cassata and R. Jaggi, Universität Bern, AKEF, Tiefenaustr.120, 3004 Bern

We studied the role of transcription factor AP-1 during mammary epithelial cell proliferation and differentiation. AP-1 is a dimer composed of Fos/Jun, Jun/Jun or ATF/Jun. The immortalized mammary epithelial cell line LU-1 shows partial differentiation and an activation of the endogenous β-casein gene in response to treatment with the lactogenic hormones hydrocortisone, insulin and prolactin (HIP). Overexpression of Fos was shown to inhibit differentiation of mammary epithelial cells *in vitro*. To study the role of different Jun proteins, stably infected LU -1 cell lines overexpressing the *jun* gene family members *c-jun, junB* and *junD* were created by the help of the retroviral vector pMV-7. For control purposes an additional viral construct containing the green fluorescent protein gene (GFP) from Aequorea victoria was also made. Cells overexpressing GFP emit green light when illuminated with blue light. This will be used as a tool to distinguish infected cells among a population of non-infected cells. Data on the phenotypic effects of the different *jun* genes and GFP on mammary epithelial cell differentiation will be presented.

S06-47

THE TRANSCRIPTION FACTOR C/EBPδ IS INDUCED BY VIP, PACAP AND NORADRENALINE IN MOUSE CORTICAL ASTROCYTES.

J-R. Cardinaux and P. J. Magistretti. Institut de Physiologie, Université de Lausanne, Lausanne, Switzerland.

High concentrations of the CCAAT/enhancer binding protein (C/EBP) family of transcription factors are found in the nuclei of fully differentiated hepatocytes and adipocytes. Therefore, it has been postulated that the regulation of energy balance may be channeled at least in part through C/EBP. Since we have previously described transcription dependent metabolic effects of VIP and noradrenaline (NA) in astrocytes (J. Neurosci. 12:4923, 1992), we have examined the effects of these neurotransmitters on C/EBP protein levels. By Western blot analysis, we observed that C/EBPδ is rapidly induced in a concentration-dependent manner by VIP [0.01-1 μM], by the VIP-related peptide PACAP [1-100 nM] or by NA [0.1-100 μM] in astrocyte cultures prepared from mouse cerebral cortex. A pharmacological analysis indicated that the effect of NA is mediated by a β-adrenergic receptor subtype. VIP, PACAP and NA therefore probably increase C/EBPδ expression via the cAMP second-messenger pathway; this was further confirmed by the induction of C/EBPδ by 8-(4Br)-cAMP or by forskolin. The protein synthesis inhibitor anisomycin at 20 μM inhibited the transmitter-induced expression of C/EBPδ at the protein but not mRNA level. These observations indicate that C/EBPδ is a cAMP-sensitive immediate-early gene in the brain. We are currently searching putative target genes whose expression is modified in response to the C/EBPδ induction.

S06-48

PYRROLIDINE DITHIOCARBAMATE DIFFERENTIALLY AFFECTS CYTOKINE- AND cAMP - INDUCED EXPRESSION OF GROUP II PHOSPHOLIPASE A_2 IN RAT RENAL MESANGIAL CELLS

Gaby Walker, Dieter Kunz, Werner Pignat*, Henk van den Bosch+ and Josef Pfeilschifter, Dept. Pharmacology, Biozentrum, Univ. Basel, Switzerland; *Res. Dept., Pharmaceuticals Div., Ciba Geigy Ltd., Basel, Switzerland and +Centre for Biomembranes and Lipid Enzymology, Padulaan 8, Utrecht, The Netherlands

Renal mesangial cells express group II phospholipase A_2 in response to two principal classes of activating signals that may interact in a synergistic fashion. These two groups of activators comprise inflammatory cytokines such as interleukin 1β (IL-1β) and tumor necrosis factor α (TNFα) and agents that elevate cellular levels of cAMP such as forskolin, an activator of adenylate cyclase. Using pyrrolidine dithiocarbamate (PDTC), a potent inhibitor of nuclear factor NFκB, we determined its role in cytokine - and cAMP - triggered group II PLA_2 expression. Micromolar amounts of PDTC suppress the IL-1β - and TNFα - dependent, but not the forskolin - stimulated group II PLA_2 activity in mesangial cells. Furthermore, PDTC inhibited the increase of group II PLA_2 mRNA steady state levels in response to IL-1β and TNFα, while only marginally affecting forskolin - induced PLA_2 mRNA levels. Our data suggest that NFκB activation is an essential component of the cytokine signalling pathway responsible for group II PLA_2 gene regulation and that cAMP triggers a separate signalling cascade not involving NFκB. These observations may provide a basis to study the underlying mechanisms involved in the regulation of group II PLA_2 gene expression.

S06-49

FUNCTIONAL ANALYSIS OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS BY TRANS-DOMINANT INHIBITION

H. Keller, M. Perroud and W. Wahli
Institut de Biologie animale, Université de Lausanne, 1015 Lausanne

Peroxisome proliferator-activated receptors (PPARs) are nuclear hormone receptors like retinoid, steroid and thyroid hormone receptors which are ligand-regulated transcription factors. PPARs are activated by natural fatty acids and by xenobiotic peroxisome proliferators such as fibrate hypolipidemic drugs and they control the transcriptional induction of several enzymes involved in the mitochondrial, peroxisomal and microsomal oxidation of fatty acids. Thus, PPARs play an important role in the regulation of fatty acid metabolism. To analyze the function of PPARs in vivo, we have chosen to study the functional knock-out of endogenous PPARs by expression of dominant negative PPAR mutants in transgenic mice. Mutant PPARs were generated that contain increasing deletions of amino acids (5-62) at the carboxy-terminus. These mutants were tested in transient transfection assays for inhibition of wild-type PPARs. One of the mutants (mouse PPARαΔ13), which lacks 13 amino acids at the carboxy-terminus, efficiently blocks transcriptional stimulation of appropriate reporter genes by wild-type PPARα, β, and γ. As a control, the related estrogen receptor is not inhibited by PPARαΔ13. This mutant will be expressed in a tissue-specific manner in transgenic mice to investigate the effects of PPAR repression in different tissues.

S06-50

EFFECTS OF PHOSPHORYLATION ON THE DNA BINDING PROPERTIES OF *XENOPUS* ESTROGEN RECEPTOR (xER).

Hihi, A.K., Claret, F-X., Mermod, N. and Wahli, W.
Inst.de Biol. Animale, UNI Lausanne, CH-1015 Lausanne, Switzerland.

The xER belongs to the steroid/thyroid hormone receptor superfamily of transcription factors. It is a nuclear protein that activates transcription after stimulation by estrogen and subsequent binding to promoters of target genes. Previous studies in our laboratory showed that either the dephosphorylation or the purification of the xER can induce the loss of its DNA binding activity. This activity can be restored with fractionated Hela cell extracts containing a DNA-activated protein kinase (DNA-PK). In this study we have asked whether the DNA-PK induces binding of the xER to its DNA response element, and whether this induction is a result of a direct effect of DNA-PK on the xER. The xER was overexpressed in a vaccinia virus system and purified by DNA affinity chromatography. Complementation experiments were then performed and DNA binding activity of the xER was tested by gel shift assays and *in vitro* phosphorylation assays.To further study DNA-PK mediated phosphorylation, we developped a Gal4-xER fusion protein approach. To facilitate purification after expression in bacteria the fusion proteins were coupled to GST. This system allowed systematic analyses of each domain of xER.

S06-51

EFFECT OF DISTINCT G-PROTEIN-COUPLED RECEPTOR KINASES ON REGULATION OF THE α_{1B}-ADRENERGIC RECEPTOR.

Diviani D., Lattion A.L and Cotecchia S. - Institut de Pharmacologie et Toxicologie, Rue du Bugnon 27, 1005 Lausanne.

The novel family of G-protein-coupled receptor kinases (GRKs) have the unique ability to recognize and phosphorylate the agonist-occupied form of G-protein-coupled receptors. βARK1 (GRK2) and βARK2 (GRK3) play a major role in agonist-induced desensitization of the cAMP response mediated by the β_2-adrenergic receptor (AR). However, very little is known about the role and receptor substrate specificity of different GRKs. Thus, we investigated the effect of different GRKs on agonist-induced regulation of the α_1BAR which is coupled to the activation of phospholipase C (PLC). The α_1BAR and its truncated mutant T368 were transiently coexpressed with different GRKs in COS-7 cells. Coexpression of βARK1 or GRK6, but not of βARK2 or GRK5, caused a two to three fold increase of agonist-induced phosphorylation of the α_1BAR, as compared to that of the receptor expressed alone. βARK1 also greatly attenuated the α_1BAR-mediated response on PLC. On the other hand, βARK1 did not impair the PLC response mediated by the T368 receptor which was unable to undergo phosphorylation. Surprisingly βARK2, wich was unable to increase agonist-induced phosphorylation of the α_1BAR, strongly impaired both the wild type and truncated α_1BAR-mediated response. Coexpression of βARK2 also induced about 40% internalization of both receptors. In conclusion, different GRKs might play a role in the regulation of the α_1BAR. However, their biochemical mechanisms might be distinct and remain to be assessed.

S06-52

CROSSTALK BETWEEN G PROTEIN-DEPENDENT RECEPTOR SYSTEMS IN NORMAL HUMAN ERYTHROID PRECURSOR CELLS

Porzig,H.,Gutknecht,R.,*Thalmeier,K.,Dept.of Pharmacology, Univ. of Bern and *GSF Forschungs-zentrum, München/D

We have analyzed G protein-mediated signalling in erythroid colony-forming cells isolated from human blood. The cells were maintained in a suspension culture that used recombinant growth factors and conditioned medium from a human bone marrow stroma cell line to allow amplification of erythroid progenitors. The cells expressed β-adrenoceptors, receptors for adenosine (A_{2B}), prostaglandins, ADP (P_{2T} and P_{2U}), platelet-activating factor, and thrombin. Adenylyl cyclase stimulation induced by adenosine and PGE_1 was potentiated by thrombin but was not affected by any of the other ligands. The effect of thrombin was blocked after inhibition of protein kinase C (PKC) or of phospholipase A_2 (PLA_2). The cyclase and G_s could both be excluded as targets for PKC-dependent phosphorylation. Therefore, an interaction of PKC with PLA_2 appears most likely. The crosstalk between thrombin and adenosine, both putative growth factors for hematopoietic cells, may represent a new regulatory mechanism in erythropoiesis.

S06-53

HSP90 IN YEAST: IT'S ROLE IN STEROID RECEPTOR AND PHEROMONE PATHWAY REGULATION

Louvion J.-F. and Picard, D.
Département de Biologie Cellulaire, Université de Genève, CH-1211 Genève 4

Unliganded steroid receptors form a complex with HSP90 via their hormone binding domain (HBD). The receptor activation involves a hormone-induced release of HSP90. Using different chimeric fusion proteins we had demonstrated that steroid HBD can also act as an autonomous regulatory cassette in yeast. This prompted us to genetically address the role of HSP90 in the steroid signal transduction in this organism by analyzing three kinds of HSP82 (the essential yeast homologue of HSP90) mutants. These mutations affect either the quantity of the protein (low versus normal levels), its integrity (point mutations or deletion analysis) or its nature (HSP82 homologues from other species). Unexpectedly, choosing STE11 (a serine/threonine kinase involved in the yeast pheromone response) as a marker protein lead to the discovery that HSP90 mutations affect the activation of the pheromone pathway. A direct interaction of HSP90 with one or more components of the pheromone pathway as well as the influence of such an interaction on the activation of the kinase cascade are being studied. As STE11 is the yeast homologue of the raf protein these observations allow us to adress the role of HSP90 in the mammalian mitogenic pathway. Interestingly, the growth inhibitory effect at elevated temperature of a dominant negative HSP82 mutant can be suppressed by osmotic stabilizers. This is reminiscent of conditions used to palliate the temperature-dependent cell lysis defect due to the absence of BCK1, a STE11 analog involved in regulating cell wall construction. Taken together these observations may attribute at least few specific cellular functions to the HSP82 chaperone in yeast.

S06-54

IDENTIFICATION AND CHARACTERISATION OF SUPPRESSORS OF *cdc16*

M. Murone, and V. Simanis, Unité de recherches sur le cycle cellulaire, Swiss Institute for Experimental Cancer Research (ISREC), Chemin des Boveresses 155, 1066 Epalinges, Switzerland

The product of the *cdc16* gene is required for correct regulation of septum formation in the fission yeast *Schizosaccharomyces pombe*. Loss of *cdc16* function causes cell cycle arrest and the production of multiple division septa without cell separation. Previous work from our laboratory has suggested that $p34^{cdc16}$ function may be required to maintain $p34^{cdc2}$ activity in mitotically arrested cells. To investigate $p34^{cdc16}$ function and regulation, we have isolated a number of suppressors of the *cdc16-116* mutation, both by transformation with a multicopy plasmid library and by selection of revertants capable of growth at 36°C following EMS mutagenesis. These experiments have identified at least three genes, two strong multicopy suppressors and an extragenic suppressor which confers cold sensitive cell cycle arrest in combination with *cdc16-116*. Characterisation of these genes and the phenotypes resulting from changes in their gene dosage and expression will be presented.

S06-55

MECHANISM OF CsA POTENTIATION OF $[Ca^{2+}]_i$ SIGNALLING IN SMOOTH MUSCLE

A. Lo Russo, A-C. Passaquin and U.T. Rüegg
School of Pharmacy, Univ. 1015 Lausanne

Drug induced local vasoconstriction appears to be responsible for the hypertensive side effect of the immunosuppressant cyclosporin A (CsA). In vascular smooth muscle cells, the Ca^{2+} response to different vasoconstrictors (measured with fura-2 or $^{45}Ca^{2+}$ efflux experiments) was increased when cells were pretreated with CsA. We have now found that inositol phosphate (Ins-P) formation is also increased in the presence of CsA, leading most likely to the potentiation of the Ca^{2+} response. Cyclosporin H and other CsA analogs devoid of immunosuppressive activity (inhibition of cyclophiline and calcineurin) were active in both, the potentiation of Ins-P metabolism and the Ca^{2+} response, indicating that there is no correlation between the immunosuppressive effect and these potentiations. *Supported by the SNSF (grant Nr. 31-36514.92) and Sandoz Pharma.*

S06-56

Measurement of serum ribonuclease levels in rheumatoid arthritis patients treated with IFN-τ

Catherine H. Schein

Laboratory for In Vitro Toxicology, Swiss Institute for Alternatives to Animal Testing, SIAT, Technopark, Pfingstweidstrasse 30, CH-8005 Zurich, Switzerland

Serum samples from a controlled blind study of the effects of human IFN-τ in 8 rheumatoid arthritic patients were obtained from Dr. M. Seitz (Inselspital, Bern). Both patients who responded to IFN-τ showed relatively low initial levels of double stranded RNA degrading enzymes (ds-RNase) in their sera, which increased after treatment. Levels of ds-RNase remained constant in the four placebo patients throughout the study period. The ds-RNase activity in sera before the start of treatment from 6 of 8 patients increased when IFN-τ was added in vitro. This demonstrates that some fraction of human ds-RNase is activated directly by IFN-τ, in a fashion analogous to the activation of bovine seminal RNase observed previously (Schein et al., 1990, FEBS Let., 270:229; Schein and Haugg, 1994, Biochem.J., in the press). Further, the two responders showed an increase after treatment in serum ds-RNase that was activated when IFN-τ was added in vitro. These results suggest IFN-τ induces a ds-RNase that it also activates. However, the importance of ds-RNase in the activity or side effects of IFN-τ will require further studies.

S06-57

ROBOTIC SYNTHESIS OF PEPTIDE AND PSEUDOPEPTIDE LIBRARIES

Boutin J.A., Hennig P., Bertin S., Lambert P.H., Volland J.P., Fauchère J.L.
Institut de Recherches Servier, 11 rue des Moulineaux, F-92150 Suresnes-Paris.

Peptide libraries have become a recognized new source of compounds for pharmacological screening. Thousands to millions of structures are produced by chemical synthesis, which can be assayed in discrete sublibraries, using the classical tools of molecular pharmacology. However, in these methods, the success of the synthesis is taken for granted and no precise information on the real composition of the synthetic libraries is available. The present work describes some advances in the analytical characterization of combinatorial libraries for the discovery of new pharmacological leads. We have designed and constructed a robot (using a Zymark rotative arm) that is able to synthesise such libraries on a large scale (24 reactors, 1g resin per reactor) using the classical methods of solid-phase synthesis and leading , e.g., to about 100mg of each of the tetrapeptide sublibraries. The building blocks are chosen among natural and non-natural amino acids. The final product, after cleavage from the resin, can be handled as an usual test compound. Mass spectrometry (MS) helped to estimate the mass distribution and to distinguish tetra- from tri- or pentapeptide libraries, while MS/MS allowed us to check bond formation even between non-natural residues. 2D-NMR was convenient to estimate the relative amino acid composition of the libraries and to quantify the 23 possible residues with respect to the known N-terminal amino acid. We also demonstrated the good reproducibility of both the synthesis and the screening process in the search of transferase-inhibiting sequences in libraries of tetrapeptides.

S06-58

CALCIUM DEPENDENT POTASSIUM AND CATION CHANNELS ACTIVATED BY BRADYKININ ON ARTERIAL ENDOTHELIAL CELLS.

BARON A., FRIEDEN M. & BENY J-L. Laboratoire de Physiologie Animale. Sciences III. Genève.
Bradykinin (BK) induces endothelium-dependent relaxation of pig coronary artery. We previously showed that endothelial cell (EC) activation by BK results in a transient hyperpolarisation approaching the K^+ equilibrium potential. Using the patch-clamp technique, we studied ionic channels activated by BK on pig coronary EC. In cell-attached configuration, 94 nM BK mainly activates two types of Ca^{2+}-dependent ionic currents whose ionic selectivity was studied in inside-out patches. In symetrical high potassium solution, we can distinguish between a high conductance potassium current, 284.9 ± 13.9 pS (n=10), and a smaller conductance cation current, 42.5 ± 1.1 pS (n=39) permeable to monovalent and divalent cations like Ca^{2+}. These Ca^{2+}-dependent channels are activated by a rise in cytosolic Ca^{2+} concentration in which both Ca^{2+} stores mobilisation and Ca^{2+} influx are thought to be involved. Opening of K^+ channels will result in the observed hyperpolarisation, whereas the Ca^{2+} influx through cation channels will contribute to the maintain of a high cytosolic Ca^{2+} level, thus triggering NO synthesis and endothelium-dependent relaxation.

S06-59

PERMEATION PROPERTIES OF A CALCIUM-BLOCKABLE MONOVALENT CATION CHANNEL OF THE ECTODERM OF THE CHICK GASTRULA

R. Sabovcik, J. Li, P. Kucera and B. Prod'hom.
Institut de physiologie, Faculté de médecine, Université de Lausanne.
The single-channel patch-clamp method is used to study the biophysical properties of a cation channel blocked by extracellular calcium located in the apical membrane of the ectoderm of the chick embryo (20 hours of incubation). The relative permeability of a series of organic cations is used to assess the size of the narrowest portion of the pore, 5.8 Angs. Organic cations larger than this value behave as blockers. Their apparent binding site is located at an electrical distance of 0.2 from the extracellular side. The modulation of the affinity of these blockers as well as of calcium by the permeant ion points to a multioccupancy mechanism of permeation. Proton blocks the sodium current with an apparent pK of 5.7. The biophysical properties of this channel are reminiscent of the L-type calcium channel and the cyclic-nucleotide gated channels.

S06-60

REGULATION OF THE pH AND PLASMA MEMBRANE POTENTIAL IN THE PARASITE *TRYPANOSOMA BRUCEI.*

C. L'Hostis, F. Quertain and J. Deshusses; Dept. of Biochemistry, University of Geneva, 1211 Geneva 4.

Two fluorescent probes, bisoxonol and BCECF, were used to monitor the plasma membrane potential and internal pH, respectively, in both forms of *T. brucei* cells. Vanadate was found to depolarize quickly bloodstream form cells by inhibiting a P-type ATPase but only in a NaCl rich buffer. It had no effect on the procyclics. Ouabain, the inhibitor of Na^+,K^+-ATPases in mammals, had no effect on the potential of trypanosomes. Quinidine, the K^+ channel opener, and veratridine, the Na^+ channel blocker, depolarized the bloodstream form cells. Analogues of amiloride, which are specific inhibitors of Na^+/H^+-antiporters, induced the depolarization of both types of trypanosomes and monitoring of the pH_i showed a rapid acidification of the cytosol upon inhibition of the H^+-extruding antiporter. Other types of pH regulation systems seem to exist in procyclics since the pH_i increased upon addition of HCO_3^- ions and a HCO_3^--independent and amiloride-insensitive system regulated the pH after a nigericin-induced H^+ influx. In general, procyclics seem able to regulate their pH and membrane potential but bloodstream form cells, which normally live in a very steady biological environment, appear to have a very limited number of regulation systems and were easily depolarized by various drugs tested.

S06-61

CAFFEINE EFFECTS ON CALCIUM RESPONSE AND STEROIDOGENESIS INDUCED BY ANGIOTENSIN II IN ADRENAL GLOMERULOSA CELLS

D. Morabito, M.B. Vallotton, A.M. Capponi, and M.F. Rossier
Division of Endocrinology and Diabetology, University Hospital, CH-1211 Geneva 14

Angiotensin II (AngII) stimulates aldosterone secretion in bovine adrenal glomerulosa cells through an activation of calcium influx into the cell. This influx is composed of two mechanisms: the capacitative influx and the activation of the voltage-operated Ca^{++} channels (VOCs). The xanthine caffeine is known to affect various elements of the Ca^{++} messenger system. Moreover, we observed that 10 mM caffeine inhibited 50% of the aldosterone secretion induced by AngII. We therefore decided to investigate the mode of action of caffeine in enzymatically-dispersed bovine glomerulosa cells.

In electropermeabilized cells or in the absence of extracellular Ca^{++}, caffeine had no effect on Ca^{++} stores nor on Ca^{++} response induced by inositol 1,4,5-trisphosphate. By contrast, we observed a significant inhibition of the sustained cytosolic free Ca^{++} concentration ($[Ca^{++}]_c$) response to AngII, which reflects calcium influx, without any notable modification in the extent of the peak response due to calcium release from intracellular stores. Caffeine appeared to act on the capacitative influx component of the Ca^{++} entry because, at 10 mM, it induced only a minimal effect on the $[Ca^{++}]_c$ response to potassium (12 mM), which depends exclusively on the activation of VOCs. This effect was not reproduced by activators of the cyclic AMP pathway such as IBMX, forskolin and Bu_2cAMP. Surprisingly, after Ca^{++} pump inhibition and store depletion by thapsigargin, caffeine had no effect on the capacitative Ca^{++} influx. These results suggest a possible positive effect of caffeine on intracellular Ca^{++} pumps.

In conclusion, in adrenal glomerulosa cells, caffeine inhibits the sustained phase of the calcium response and the steroidogenesis induced by AngII by a mechanism that might involve the Ca^{++} pump of intracellular stores.

S06-62

INCREASED CYTOSOLIC CALCIUM IN DYSTROPHIN-LACKING MYOTUBES UNDER STRESS

Leijendekker WJ, Passaquin A-C, Rüegg UT
School of Pharmacy, UNIL, 1015 Lausanne

The free intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) was measured (Fura-2) in single myotubes from mice lacking dystrophin (C57BL/*mdx*), an animal model of Duchenne muscular dystrophy. Resting $[Ca^{2+}]_i$ was similar in control and *mdx* myotubes (41 nM and 40 nM, resp.). A 250% increase in $[Ca^{2+}]_i$ was observed in *mdx* myotubes exposed to 40 mM extracellular $[Ca^{2+}]$, whereas only a 40% increase occurred in controls. Exposure of *mdx* myotubes to a hypoosmotic solution (75 mOsm) resulted in a 2.5-fold increase in $[Ca^{2+}]_i$ compared to 1.5-fold in controls. These results indicate that under stress, i.e. at high extracellular $[Ca^{2+}]$ or low osmolarity, myotubes of *mdx* mice exhibit an impaired calcium handling.

Supported by the Swiss Foundation for research on Muscular Diseases

S06-63

Ca^{2+} INFLUX INDUCED BY AGONISTS AND POOL DEPLETION IN VASCULAR SMOOTH MUSCLE CELLS.

Skutella M and Rüegg UT, Univ. of Lausanne
School of Pharmacy, 1015 Lausanne.

Ca^{2+} influx in vascular smooth muscle cells (A7r5 cell line) was induced by [Arg[8]] vasopressin (AVP) or the Ca^{2+}-ATPase blocker thapsigargin (TG). Both stimuli showed equal magnitudes of maximal stimulation of $^{45}Ca^{2+}$ influx. Various agents of different chemical structure (DIF-1, L-651,582, econazole, SK&F 96365 and LOE 908) inhibited influx induced by both stimuli with similar potency. The same agents also inhibited contractions of rat mesenteric arteries induced by these stimuli. These results suggest the existence of one common pathway for Ca^{2+} influx induced by AVP and TG. It is very likely that this is due to activation of CRAC (Calcium release activated channel), the channel which is stimulated by depletion of the Ca^{2+} pools.

Supported by SNSF (grant Nr. 31-36514.92)

S06-64

The JAK protein tyrosine kinases in signal transduction

Andrew Ziemiecki, Peter Küng, Gisela Zürcher and Anne-Catherine Andres AKEF, Department of Clinical Research, CH-3004 Bern

Protein tyrosine kinases (PTKs) are integral components of the cellular machinery responsible for the transduction and processing of many extra- and intra-cellular signals. The Janus kinase (JAK) family of intracellular PTKs are characterized by the possession of a second tyrosine kinase related domain located N-terminal to the bona fide kinase domain and five additional domains of homology referred to as JAK homology or JH domains whose function(s) are presently unknown. An important breakthrough in the understanding of the role played by this family of PTKs in signal transduction came from the elegant demonstration that the JAK family kinase TYK2 is required for signal transduction initiated by interferon α/β. Subsequently, many members of the cytokine receptor superfamily have been shown to compensate their lack of a tyrosine kinase activity by utilizing members of the JAK family in various combinations for signal transduction. Common to many of these signalling pathways is the involvement of a family of cytosolic transcription factors, the Signal Transducers and Activators of Transcription (STATs). Members of the JAK family are either preassociated with or associate after ligand stimulation with the cytokine receptors. In both cases, ligand stimulation leads to tyrosine phosphorylation of the receptor/JAK complex and recruitment of STAT proteins which in turn are activated by tyrosine phosphorylation (by the JAKs?) and translocate to the nucleus to regulate gene expression.

S06-65

The role of the $p70^{s6k}/p85^{s6k}$ signalling pathway and S6 phosphorylation in mitogenesis

George Thomas, Friedrich Miescher Institute, Basel

The $p70^{s6k}/p85^{s6k}$ represent two isoforms of the same kinase which are encoded by a common gene and are located either in the cytoplasm, $p70^{s6k}$, or in the nucleus, $p85^{s6k}$. Recent studies show that both isoforms lie on the same signalling pathway which is distinct from the $p21^{ras}/p42^{mapk}$ signalling pathway. Indeed the $p21^{ras}/p42^{mapk}$ and the $p70^{s6k}/p85^{s6k}$ signalling pathways bifurcate at the level of specific SH2-docking sites within the insert kinase domain of the PDGF-receptor. The intracellular target of the of the $p70^{s6k}$ is 40S ribosomal protein S6 whose phosphorylation has been implicated in the selective translational upregulation of a family of mRNAs. Consistent with this hypothesis it has recently been demonstrated that the highly tauted immunosuppressent rapamycin, which specifically blocks $p70^{s6k}$ activation and increased S6 phosphorylation, also blocks the upregulation of the same family of mRNAs. The family is characterized by a polypyrimidine tract at its 5' transcriptional start site and is present in a number of translational components including ribosomal proteins and elongation factors. These are essential gene products whose halt in synthesis will block cell growth, and thus would explain the mechanism by which rapamycin inhibits cell growth.

Developmental neurobiology

S07-01

BLOOD VESSEL FORMATION IN THE AVIAN NEURAL TUBE

Kurz, H., Christ, B., Gärtner, T., Anatomisches Institut, Lehrstuhl II, Albert-Ludwigs-Universität, Albertstr. 17, D-79104 Freiburg, Germany

According to most earlier reports, the avian neural tube (NT) is vascularized only by ventral sprouts. This view is modified and extended by our observations on quail embryos and chick-quail chimeras. With the monoclonal, anti-quail haemangioblast antibody QH1, angioblasts in the cervical quail NTs were detected first dorsally as single, migrating cells. They populate the NT at random intervals, without correspondence to the somitic segmentation, and with bilaterally asymmetric distribution. Only after this initial dorsal immigration, sprouts are formed independently in the ventral NT, originating from both primitive arterial tracts. These endothelial sprouts penetrate into the NT between the floor plate and the future motor column. The site of the dorsal invasion corresponds to the paths of neural crest cells. Both sources of endothelial cells contribute to intraneural vessel plexus. Interactions with the neuroepithelium and the extracellular matrix are discussed.

S07-02

Positional determination of the naso-temporal retinal axis coincides with asymmetric expression of proteins along the anterior-posterior axis of the eye primordium.

S. Thanos, E. Hummler and J. Mey.

We used the topographic projection of retinal ganglion cells onto the optic tectum, to analyse experimentally altered patterns of retinotectal connectivity and to examine molecular asymmetries in the tissue during the stage of positional specification. The first goal was achieved by removing parts of the presumptive temporal primary optic vesicle at stage 11 (40 to 45 hours of incubation) and shifting of tissue with presumptive nasal properties into the wound during the events of wound-healing. Participation of the shifted tissue to the healing resulted in assembly of a temporal retina with mosaic-like projection properties, as examined by retrograde double staining of the retinal ganglion cells from the optic tectum. Besides of cells with normal temporal-rostral projections, clusters of ganglion cells with nasal-like projection identities appeared labelled within temporal hemiretina. The number of clusters increased with the amount of resected tissue, and by almost complete ablation of the presumptive temporal anlage, a temporal hemiretina with predominantly nasal retinotectal specificity was created. These neuroanatomical results suggested that neuroepithelial cells had fixed nasal and temporal positional specificities at the stage of ablation.
To examine differences in the cells derived of either half of the eye cup, we performed biochemical one- and two-dimensional gel electrophoresis of the hemianlagen at stage 11. In addition, incorporation of ^{35}S-methionin into newly synthesized peptides was investigated. Both techniques revealed the exclusive expression of one major and 3 less abundant proteins within the presumptive nasal anlage. The most abundant of these proteins has a molecular weight of about 40kD and is clearly distinguishable both in gel electrophoresis and autoradiography. The assymetric protein patterns had disappeared when the retina was analysed with the same methods at the more advanced embryonic days E4 and E6. The asymmetry in the expression of proteins in the retinal primordium maybe the biochemical correlate of an early positional specification of the retinal neuroepithelium. The difference in the protein expression may explain that mixing of the positionally specified cells of either origin results in projection mosaics.

S07-03

DEVELOPMENT OF THE FUNCTIONAL ARCHITECTURE OF THE MAMMALIAN VISUAL SYSTEM: ROLE OF AXONAL GROWTH AND ITS CONTROL

Christian M. Müller, Claudius Griesinger, Sabine Kindl & Michaela Schweizer,
Max-Planck-Institute for Developmental Biology, 72076 Tübingen, Germany

One basic feature of the central nervous system is the presence of functional organizations containing ordered representations of stimulus features relevant for sensory processing. In the mammalian visual system there is a high topographic order with respect to the location of receptive fields (retinotopy), the ocularity of neuronal responses (ocular dominance layers/stripes), and the orientation selectivity of cortical cells (orientation columns). The neuronal response specificities rely on very accurate axonal connectivities which elaborate in an activity-dependent manner from exuberant axonal arborizations during a restricted 'critical period' in postnatal development.
Experimental evidence will be summarized indicating that adaptive plasticity is due to rapid morphological changes including axon- and synapse-elimination and axon growth. Furthermore, it will be shown that morphological, axonal plasticity in the developing visual pathway can be influenced by the glial environment.
The expression of the astroglial derived matrix molecule tenascin reveals a close correspondence with the developing layering of the thalamic visual relay, the dorsal lateral geniculate nucleus. This is compatible with an instructive role of this molecule on layer formation by an influence on axonal growth. Evidence will be presented showing that an oligodendroglial protein contributes to the end of the critical period for cortical plasticity by inhibiting axonal growth.
The data will be discussed with respect to the role of axonal growth and retraction in the formation of functional topopgraphies in the CNS.

S07-04

Identification of protein tyrosine phosphatases that are expressed in the peripheral nervous system

Dirk H-H. Neuberg, Beat Schweitzer, Josef P. Magyar and Ueli Suter.
Institute of Cell Biology, Swiss Federal Institute of Technology, ETH Hönggerberg, CH-8093 Zurich

The peripheral nervous system (PNS) provides an excellent experimental system to study recurring themes in the development of multicellular organisms like cell-cell interactions in which one cell determines the fate of its neighbour. This is exemplified by PNS myelination where the two cell types involved, neurones and Schwann cells, exhibit a profound influence on each other. While axons regulate myelination and proliferation of Schwann cells, Schwann cells in turn are the main determinants of axon calibre and sodium channel distribution along axons. As an important experimental advantage, these differentiation processes are mostly reversible and can be studied during nerve degeneration and regeneration.
To examine the potential impact and role of protein tyrosine phosphatases (PTPs) in these processes, we designed ambiguous primers based on alignments of the catalytic domains of the PTPs. Using these primers, we performed nested RT-PCR on RNA from rat sciatic nerve and total RNA isolated from cultured rat Schwann cells.
The resulting different PTP cDNA fragments were pooled and used as a probe for a low stringency screening of a cDNA-library derived from postnatal day 6 rat sciatic nerve. Using this approach several different PTP-clones have been identified, including novel and known PTPs expressed by glial cells.
A first analysis of the expression pattern of the found PTPs will be performed by Northern blot analysis and in situ hybridisation. PTPs that show an interesting pattern of cell type specific expression and regulation will be selected for detailed analysis.

S07-05

NR-CAM/BRAVO IS A NONNEURONAL RECEPTOR FOR THE NEURONAL CELL ADHESION MOLECULE AXONIN-1

D.M. Suter, G.E. Pollerberg*, A. Buchstaller, and P. Sonderegger. Institute of Biochemistry, University of Zurich, CH-8057 Zurich, Switzerland. *Max-Planck-Institute for Developmental Biology, Department of Biochemistry, D-72076 Tübingen, Germany

Immunoglobulin superfamily molecules are involved in a number of cell - cell and cell - matrix interactions during the development of the nervous system. During neurite outgrowth axonin-1 functions as a substratum for growth cones expressing Ng-CAM with which it undergoes a heterophilic interaction. Here we report that axonin-1 also interacts with Nr-CAM/Bravo. Binding of axonin-1 to molecules other than Ng-CAM was found when fluorescent beads carrying covalently coupled axonin-1 bound to glial cells of cultured chicken dorsal root ganglia (DRG) in an interaction that could be perturbed only by anti-Nr-CAM antibodies. Immunostaining and *in situ* hybridization revealed that these DRG glial cells indeed express Nr-CAM. Anti-axonin-1 or anti-Nr-CAM Fab fragments perturbed the formation of close contacts between neurites and glial cells in culture, indicating a role of the binding between axonin-1 of the neurite membrane and Nr-CAM of the glial cells for the early phase of axon ensheathment.

S07-06

F11/AXONIN-1 RELATIVES IDENTIFIED BY PCR

Rader, C., Giger, R.J., Euringer, M., Jäckli, P.A., and Sonderegger, P.
Biochemisches Institut der Universität Zürich, CH-8057 Zürich

Axonal cell adhesion molecules (AxCAMs) composed of repeated immunoglobulin- and fibronectin-type-III-like domains are thought to play a crucial role in the developing or regenerating nervous system. Based on structural criteria, the Ig/FNIII-like AxCAMs can be subdivided into two groups. In the chicken, one group includes the GPI-anchored proteins F11 and axonin-1, the other the transmembrane proteins NgCAM, NrCAM, and neurofascin. To identify novel members of the F11/axonin-1 group, degenerate PCR primers based on conserved amino acid sequences in F11, axonin-1, and their mammalian relatives were synthesized. RT-PCR was carried out on mRNA purified from embryonic and postnatal chicken brain. Prior to a second amplification, PCR products derived from F11 and axonin-1 were eliminated by selective digestion. Subcloning and sequencing revealed novel sequences with high homology to F11 and axonin-1.

S07-07

EFFECTS OF L-SERINE ON NEURONS *IN VITRO*

Reto SAVOCA, Urs ZIEGLER and Peter SONDEREGGER
Institute of Biochemistry, University of Zurich, CH-8057 Zurich

We have examined the influence of amino acids on chicken embryonic dorsal root ganglion neurons *in vitro* and found that serine has a marked impact on the phenotype of the developing neurites. In cultures supplemented with serine at micromolar concentrations (10-200 μM), the neurites grew up to 100 % longer and developed a more complex branching pattern. The observed effects of L-serine were concentration-dependent, stereospecific and found on several different substrata, such as laminin, Ng-CAM and axonin-1. Similar observations were made with neurons of the central nervous system, such as embryonic retinal explants. Addition of other amino acids to dorsal root ganglion neurons had no effect. We conclude that serine, although it belongs to the group of non-essential amino acids and is not known as a neurotransmitter, is an important factor for the morphological differentiation of neurons *in vitro* and recommend to add serine to the culture media.

S07-08

FUNCTIONAL CHARACTERISTICS OF THE MAP2 REPEAT DOMAIN

B. Ludin, U. Fünfschilling and A. Matus
Friedrich Miescher Institut, Basel

The neuron-specific microtubule-associated protein MAP2 promotes microtubule assembly in vitro and induces bundling of microtubules and formation of processes when expressed in non-neuronal cells. MAP2 interacts with microtubules through its C-terminal domain which contains imperfect repeats of a 31 amino acid sequence motif. It has been proposed that the repeats constitute individual microtubule-binding domains.

Are the repeats functionally equivalent units? What function is conferred by the repetition? How does MAP2 mediate microtubule bundling? In order to investigate these aspects of MAP2 function we have produced several repeat-domain-derived peptides as well as MAP2 mutants retaining only 1 or 2 of the originally 3 or 4 repeats and studied their effects on microtubules in vitro and in cultured cells.

S07-09

DENDRITIC TARGETING OF MAP2 IN NEURONS OF TRANSGENIC MICE.

K. Marsden, J. Ferralli, T. Doll, F. Botteri and A. Matus, Friedrich Miescher-Institut, P.O.Box 2543, 4002, Basel.

The neuron-specific microtubule protein MAP2 occurs in distinct adult (MAP2) and embryonic (MAP2c) forms. MAP2c is only present during development, linking its expression to neuronal morphogenesis. To investigate its potential role we have produced transgenic mice that express MAP2c in adult brain neurons. In one line brain MAP2c levels are higher than those of endogenous adult MAP2. Despite this there is no detectable change in neuronal morphology, suggesting that MAP2c function is regulated by signals in the developing brain. Interestingly transgenic MAP2c is limited to neuronal dendrites, both in brain sections and in hippocampal cell cultures. Since our transgene construct is devoid of untranslated sequences these results place the putative MAP2 dendritic targeting signal somewhere within the 1.4 kb MAP2c coding sequence. This is a significant reduction compared to previous results, on whose basis the signal could have been located anywhere within the 9 kb of the adult MAP2 mRNA sequence.

S07-10

Lissencephaly Gene (*LIS1*) Expression in the CNS suggests a Role in Neuronal Migration

U. Albrecht, O. Reiner#~, C. Wong, A.M. Buchberg*, C.T. Caskey# and G. Eichele
*Dept. of Biochemistry and #Dept. of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX 77025 USA ~Dept. of Molecular Genetics and Virology, Weizmann Institute, Rehovot 76100, Israel *Jefferson Cancer Institute, Philadelphia, PA 19107 USA.*

Miller-Dieker lissencephaly syndrome (MDS) is a human developmental brain malformation caused by neuronal migration defects resulting in abnormal layering of the cerebral cortex. *LIS1*, the gene defective in MDS, encodes a subunit of brain platelet activating factor (PAF) acetylhydrolase which inactivates PAF, a neuroregulatory molecule. We have isolated murine cDNAs homologous to human *LIS1* and mapped these to three different chromosomal loci. The predicted sequences of murine Lis1 protein and its human homolog LIS1 are virtually identical. In the developing mouse and human, *Lis1* and *LIS1* genes were strongly expressed in the cortical plate. In the adult mouse *Lis1* transcripts were abundant in the cortex and hippocampus. The direct correlation between cortical defects in MDS patients and *Lis1* expression in the murine cortex suggests that the mouse is a model system suitable to study the mechanistic basis of this intriguing genetic disease.

S07-11

LESION OF THE ZEBRAFISH OPTIC NERVE INDUCES NEURONAL GENES INVOLVED IN REGENERATION.

Bormann, P., Zumsteg, V., and Reinhard, E. Dept. of Pharmacology, Biozentrum, University of Basel, 4056 Basel

Retinal ganglion cells (RGCs) in the adult zebrafish (*danio rerio*) retina are easily accessible for studies of regeneration in the adult central nervous system. RGCs that are deprived from their target, e.g., after injury of the optic nerve, start to re-express genes that were selectively silenced after neuronal development had finished. One of these genes that is primarily expressed in neurons extending processes is the growth-associated protein GAP-43. *In situ* hybridization studies showed that a subset of RGCs start to express GAP-43 mRNA as early as 24 hours after lesion (a.l.) of the optic nerve. Virtually all RGCs express GAP-43 at maximal levels between 6 and 10 days a.l. Expression is decreased thereafter and is below detection limit 3 weeks a.l. Downregulation of GAP-43 corresponds with the time when regenerating axons reach the optic tectum and establish new synapses.

To isolate additional genes that are specifically expressed in regenerating neurons and, therefore, might be involved in neurite outgrowth, we started to compare gene expression in regenerating and non-regenerating retinas by the method of Differential Display.

S07-12

MOLECULAR AND GENETIC ANALYSIS OF *C. ELEGANS* HOMEOBOX GENES

Bürglin, T.R., and Ruvkun, G.* Biozentrum der Universität Basel, and * Dept. of Genetics, Harvard Medical School, Boston

ceh-6 is a member of the POU-III homeobox family. It is expressed in 10 bilaterally symmetric neurons in the brain (RMDD, RMDV, AUA, AVH, SABV), the excretory cell (osmoregulation), the Pn.a neuroblasts in the ventral nerve cord, and the Y, K, F, B, U cells in the rectum. We generated a knock-out mutation in *ceh-6*. 80% of the animals die during morphogenesis, when the rectum ruptures. The other 20% die during larval stage 1, consistent with a defect in the excretory cell. *ceh-20* is highly similar to the human homeobox protooncogene *PBX1*. During early embryogenesis the gene is expressed only in the posterior half of the embryo. During larval stages *ceh-20* is most strongly expressed in the ventral nervecord, the retrovesicular ganglion and other neuronal structures in the body. Weaker staining is seen in the hypodermis and muscle cells. Recently, mutations in this gene have been isolated in Dr. Stern's laboratory, and consistent with the expression pattern, defects in the central body region are observed.

S07-13

MODULATION OF THE LAMINAR DEVELOPMENT OF THE MOUSE SOMATO-SENSORY CORTEX BY MONOAMINES.

HORNUNG J.P. and OSTERHELD-HAAS M.C., Institute of Anatomy, University of Lausanne, 1005 Lausanne.

The mouse cerebral cortex develops from a trilaminar sheet into the mature six-layered cortex over the first postnatal month following a precise temporal pattern. In the present study, we wanted to evaluate the influence of neonatal monamine deprivation on the rate of maturation of the cerebral cortex, measured in terms of laminar differentiation, in three groups of animals: control, 5-7-dihydroxytryptamine-injected (depleted of all monoamines), and 6-hydroxydopamine-injected (depleted of catecholamines only) mice. Although there was no difference in laminar thickness at one month postnatal between depleted and control animals, the pattern of laminar maturation in depleted animals was delayed as compared to the controls. The differentiation of layer IV was delayed by 2-3 days at the end of the first postnatal week. The size of the upper granular layers was markedly reduced at the end of the second week, and increased rapidly towards control values during the third week. Both neurotoxin treatments had a similar effect, but the growth retardation was larger after monoamine than after catecholamine depletion. This asynchrony in development of cortical neurons could result in subtle alterations of cortical connectivity, which is stabilized in part by functional interconnections. Supported by SNF grant 31-30842.91.

S07-14

DEVELOPMENTAL AND REGIONAL EXPRESSION OF 5α-REDUCTASE mRNA IN THE RAT BRAIN

Lauber M.E. and Lichtensteiger W., Institute of Pharmacology, University of Zürich.

Sex steroid hormones play a crucial role in many aspects of development and function of the brain. Whereas the role of estrogens has been examined extensively, the physiological importance of androgens in the brain is quite unclear. In the rat model, it is generally agreed that androgens are involved in the regulation of food intake and are required for the complete manifestation of a number of sex differences. Testosterone and dihydrotestosterone represent the major androgens in the central nervous system, the former is produced in the gonads and reaches the brain *via* the blood system, the latter results from *in situ* enzymatic 5α-reduction of testosterone in certain regions of the brain. We examined the expression of mRNA encoding 5α-reductase (type 1) in the brain of rats at different stages of development by means of *in situ* hybridization. We found that mRNA specific for this enzyme is expressed in the fetal brain at high levels during the last week of gestation. The regional localization mainly included neuroepithelial cells of the retina, the caudate putamen and the cortex, but also the trigeminal nerve expressed 5α-reductase mRNA. At early postnatal stages, 5α-reductase mRNA expression was decreased in the neuroepithelial regions, but was found at high levels in the hippocampus and some regions of the thalamus. In the adult animal, 5α-reductase mRNA expression appeared to be shifted from the neuroepithelial regions to white matter structures such as the corpus callosum, the optic chiasm, the lateral olfactory tract and the stria medularis thalamus.
Our results indicate that androgens, or 5α-reduced metabolites of other steroid hormones (like progesterone), might serve a role in proliferation and growth of neurons in perinatal brain development. In addition, our postnatal observations support the notion that 5α-reduced steroids might be involved in the process of myelinization.

S07-15

Evolution of CK Genes and their Expression in Chicken Tissues and Embryonic Development

S.M. Mühlebach, T. Wirz, U. Brändle and J.-C. Perriard
Instiut for Cell Biology, ETH Hönggerberg, CH-8093 Zürich

The gene family of the creatine kinases consists of four known genes. Two of the resulting proteins localize to the cytosol (termed M- and B-CK) and two to the intermembrane space of mitochondria (termed Mib- or sarcomeric Mi-CK and Mia- or ubiquitous Mi-CK (a and b = more acidic, resp. basic pI)).
We have cloned cDNA and the gene of the chicken Mia-CK, compared its protein sequence to the 26 kown CK-proteins and its gene structure to the 9 kown CK-genes. The comparison of the protein sequences allowed us to define a CK-framework of regions highly conserved between all CKs and of "diagnostic boxes" conserved within an isoform species. Together with the conserved gene structures of Mi-CKs and cytosolic CKs, respectively, we determined a tentative evoutionary tree of CKs, which supports the idea that two geneduplication events are at the origin of the four different genes.
Analysis of expression of the four CK-genes in different adult tissues indicates that tissues with high and fluctuating energy demand rely on a functional PCr-shuttle with the expression of a cytosolic and a mitochondrial CK. However, in embryonic development the Mi-CKs appear late while the cytosolic CKs are present very early, indicating independent regulation.

S07-16

CALCIUM-INDUCED DISRUPTION OF MICROFILAMENTS IN LEECH GROWTH CONES: GELSOLIN A POSSIBLE MEDIATOR ?

M.D. Neely, E. Macaluso and J.G. Nicholls, Biozentrum Univ. Basel, 4056 Basel, Switzerland

Depolarization of leech neurons leads to growth cone collapse and neurite retraction, a response mediated by the influx of calcium (S. Grumbacher-Reinert and Nicholls, 1992, J. Exp. Biol. 167:1-14; Neely, 1993, J. Neurosci. 13:1292-1301). These changes in growth cone morphology and motility are accompanied by a loss of microfilaments (Neely and Gesemann, J. Neurosci., *in press*). The mechanism by which elevations in intracellular calcium concentrations leads to disruption of the microfilament organization is not known for neurons or other types of cells, in which similar observations were made. Gelsolin is a microfilament-associated protein which severs actin-filaments upon activation with calcium. We have examined the possible presence of gelsolin in leech growth cones using two different antibodies. We observed an antigen that is very abundant in the filopodia and especially prominent in their distal regions, where we do not detect abundant microfilaments. In the lamellipodia it co-localizes with microfilaments. This distribution makes this antigen a good candidate for a regulator of microfilament structure during neurite extension and retraction. We are currently analyzing its localization in growth cones after influx of calcium. In addition we are examining its presence in the regenerating CNS of the leech and studying its biochemical properties.
This work was supported by a grant from the Swiss Nationalfond No. 3127814.89 to J.G. Nicholls.

S07-17

BETA-GALACTOSIDASE EXPRESSION REGULATED BY TYROSINASE 5'SEQUENCE DURING EMBRYOGENESIS IN TRANSGENIC MICE

Tief,K.,Schmidt,A.,Beermann,F., Swiss Institute for Experimental Cancer Research (ISREC), CH-1066 Epalinges

Melanocytes are responsible for the visible pigmentation and derive from two distinct developmental origins. The melanocytes found in hair bulb and epidermis originate in and migrate from the neural crest. The pigment cells that form the pigmented retinal epithelium of the eye differentiate from the neuroectoderm. Tyrosinase, the essential enzyme for melanin production, has been described as a melanocyte-specific protein. We were interested in the expression pattern of the corresponding gene and the distribution of melanocytes during mouse embryogenesis. We used promoter lacZ constructs to produce transgenic mice which allowed a highly sensitive detection of promoter actifity in whole mount embryos and sections.
From day 10.5 of embryogenesis onwards we obtained lacZ staining in various tissues which are not known to contain melanocytes. We are now further investigating the origin of these cells.

S07-18

METABOLIC INTERACTIONS AND SIGNALS TRAFFICKING BETWEEN GLIAL CELLS AND NEURONS

M. Tsacopoulos and A.-L. Veuthey, Experimental Ophthalmology Laboratory and Department of Physiology, Medical Research Center, Geneva.

The honeybee retina is a nervous tissue with crystal-like structure in which glial cells and photoreceptor-neurons constitue two distinct metabolic compartments. Glial cells transform glucose to alanine and, with proline, fuel the mitochondria of the photoreceptors. Proline supplies the Krebs cycle by making glutamate and, in turn, α-ketoglutarate. Light stimulation of the photoreceptors causes 200% increase of their oxygen consumption and 50% decrease of the pool size of proline and of glutamate. The use of proline induces a light-dependent rise of ammonia in the extracellular space of the superfused retina measured with microelectrodes and enzymatically. Alanine formation fixes NH_3 at a rate far exceeding glutamine formation. This is consistent with the rise of a glial pool of alanine upon photostimulation. The finding showing a substential ^{15}N-alanine formation from ^{15}N-proline strongly suggest that ammonia and possibly glutamate are transferred from photoreceptors to glial cells. In the glia two predominantely cytosolic enzymes, glutamate-dehydrogenase and alaline amino-transferase, work in tandem. The increase in NH_4 results in an increase in glutamte production which in turn activates the formation of alanine. The consequence of this activation by NH_4 is an increase of NAD^+ available for glycolysis. Indeed, stimulation of photoreceptors induce an increase of glycolysis in the glia.

S07-19

THYROID HORMONE PROMOTES SURVIVAL AND NEURITE OUTGROWTH OF PRIMARY SENSORY NEURONS IN RAT DORSAL ROOT GANGLION CULTURES

I. Barakat Walter, Institut d'Histologie et d'Embryologie, Faculté de Médecine, Rue du Bugnon 9, CH-1005 Lausanne

Thyroid hormone could be one of several factors which are involved in development and regeneration of the peripheral nervous system. The action of thyroid hormone on responsive cells is necessarily mediated through specific receptors (T_3R). In the present study we showed that : 1) primary sensory neurons and Schwann cells possess T_3R in dorsal root ganglion cell cultures; 2) triiodothyronine (T_3) in physiological concentration enhances neuronal survival and fibre outgrowth of sensory neurons in culture. In fact, in dissociated DRG cell cultures T_3 stimulates the survival of sensory neurons in mixed as well as in neuron-enriched cell culture in the absence of exogenously added NGF or other neurotrophic factors. In explant DRG cell cultures, T_3 enhances neurite outgrowth of sensory neurons. This stimulation is practically abolished when cultures are treated with mitotic inhibitor cytosine arabinoside or with anti-NGF antibodies. This results suggest that T_3 stimulates neuronal survival by a direct effect on neurons, while stimulation of fibre outgrowth by T_3 may be mediated through non neuronal cells (S.N.F. N° 3367-92)

S07-20

STEEL FACTOR ACTIVITY DIRECTS MELANOCYTE PRECURSOR DISPERSAL ONTO THE LATERAL MIGRATION PATHWAY

Wehrle-Haller, B. and Weston, J.A., Institute of Neuroscience, University of Oregon, Eugene, OR 97403

Trunk neural crest cells segregate from the neuroepithelium and enter a "migration staging area" (MSA) lateral to the embryonic neural tube. After some crest cells have begun to migrate on the medial pathway, a subpopulation of crest-derived cells remain in the MSA that express mRNAs for the receptor tyrosine kinase, c-kit, and the tyrosinase related protein (TRP-2). These cells, putative melanocyte precursors (MPs), then migrate on a lateral pathway toward the epithelial dermatome, and subsequently disperse into nascent mesenchyme. MPs transiently require the c-kit ligand, Steel factor (SLF), for survival, and SLF mRNA is known to be transiently localized in the dorsal epithelial dermatome before the onset of trunk crest dispersal on the lateral pathway. To assess the role of SLF function in MP dispersal and fate on the lateral pathway, we analysed several different SLF mutants for altered MP behavior. For example, *Sl* is a null mutation that lacks SLF, whereas Sl^d lacks cell surface-associated SLF but produces a soluble form of SLF. No MPs were detected in the dermatome of embryos homozygous for the *Sl* allele, whereas in embryos homozygous for the Sl^d allele, MPs initially disperse on the lateral pathway, but were not detected in the dermis later in development. We conclude, that soluble SLF is required for the onset of MP migration toward the dermatome, whereas membrane bound SLF plays a role in MP survival in the dermis. Supported by EMBO ALTF 169-93 (BW-H) and NIH DE-04316 (JAW).

S07-21

CHARACTERIZATION OF FULL-LENGTH CHICK AGRIN, A BASAL LAMINA PROTEIN INVOLVED IN THE FORMATION OF SYNAPSES

Denzer, A.J., Gesemann, M., and Rüegg, M.A., Dept. of Pharmacology, Biozentrum, University of Basel, 4056 Basel

Agrin is a protein of the extracellular matrix with a calculated molecular weight of ~ 220 kD that induces the aggregation of acetylcholine receptors (AChRs) and other molecules concentrated at the neuromuscular synapse. As described in the accompanying abstract by Gesemann et al., this activity is contained in a 21 kD, C-terminal fragment and is strongly affected by alternative mRNA splicing. To find additional functions for agrin, we have started to characterize full-length agrin. Primer extension studies and characterization of the agrin gene revealed a ~ 300 bp long extension at the 5' end to the cDNA sequence published for chick agrin. When transiently expressed in COS cells, recombinant full-length agrin is secreted from COS cells and migrates on SDS-PAGE with an apparent molecular weight between 400 and 600 kD. Deglycosylation experiments show that agrin synthesized by transfected cells is a heparan sulfate proteoglycan. With polyclonal antisera against recombinant agrin we show by Western blots that agrin *in vivo* has also an apparent molecular weight between 400 and 600 kD. In addition, we have found novel sites at the N-terminal part of agrin that undergo alternative mRNA splicing. In particular, a 7 amino acid long insert seems to influence the way agrin is associated with cells. We are currently investigating the tissue specificity of the novel splicing sites and their physiological significance in the formation of synapses.

S07-22

SITES OF CHICK AGRIN REQUIRED FOR HEPARIN-BINDING AND FOR INDUCTION OF POSTSYNAPTIC SPECIALIZATIONS ARE DISTINCT

Gesemann, M., Cavalli, V., Denzer, A.J., and Rüegg, M.A., Dept. of Pharmacology, Biozentrum, University of Basel, 4056 Basel

Agrin has originally been described as a 95 kD fragment, isolated from basal lamina extracts of the electric organ of the marine ray, that induces the aggregation of acetylcholine receptors (AChRs) on muscle cells. Several lines of evidence strongly suggest that this activity is mediated by a receptor that activates an intracellular signaling cascade. Cloning of agrin has revealed that it is expressed in several isoforms that arise from alternative mRNA splicing. While the neural isoform is active at picomolar concentrations, even 100 nM of the muscle isoform fail to induce clustering. We have now mapped the active site to a 21 kD, C-terminal fragment of agrin. However, when compared with the 95 kD fragment, its activity is lowered by more than two orders of magnitude. Unlike the 95 kD fragment, this 21 kD fragment neither binds to heparin nor does heparin inhibit its capability to induce AChR aggregation. Hence, agrin domains required for binding to heparin and those for AChR aggregation are distinct from each other. Although these results show that the heparin-binding site of agrin is not necessary to activate the signal-transducing agrin receptor, the much lowered activity of the 21 kD fragment suggests that the heparin-binding site may support AChR-aggregating activity by conferring binding to a helper protein. A strong candidate for such a co-receptor is α-dystroglycan that has been shown to bind agrin and to accumulate at agrin-induced AChR aggregates.

S07-23

ELECTROPHYSIOLOGICAL CHARACTERIZATION OF NICOTINIC RECEPTOR (AChR) SUBTYPES ON RAT SUPERIOR CERVICAL GANGLION (SCG) NEURONS

Britt, J.C., Brenner, H.R., Dept. of Physiol., Univ. of Basel

The properties of native nicotinic AChRs in rat SCG neurons maintained in short term culture (up to 60 hours) were analyzed. The nicotinic agonist DMPP was pressure ejected onto isolated neurons or excised membrane patches for 1-30s. DMPP-induced whole cell currents reached peak amplitude within 330ms (S.E.=±6ms, n=312) and then declined. Peak current amplitudes varied from 120pA to 3180pA ($\bar{x}$=1055pA S.E.=±62pA, n=111) and were only weakly correlated to cell capacitance. The rate of whole cell current decay during constant DMPP application varied between neurons. Half decay times ranged from 0.38s to 11s with a mean of 2s (S.E.=±0.17s, n=113). By exchanging intracellular Cl^- by Cs^+methanesulfonate, the possibility of interference by a Ca^{++} activated Cl^- current was excluded. The differences in half decay time are due to different functional properties of AChR subtypes, as indicated by currents recorded from outside-out patches upon rapid agonist application (2ms). The results indicate the presence of two distinct AChR subtypes on SCG neurons, one with a fast and one with a slow activation/desensitization rate but with similar single channel conductances. In addition evidence for a third AChR subtype with lower single channel amplitude and different I/V-relation was obtained. No evidence was found for developmental changes in the functional properties of AChRs from fetal to early adult stages.

S07-24

CHANGES INDUCED IN ORGANOTYPIC HIPPOCAMPAL CULTURES AFTER LESION OF SCHAFFER COLLATERALS.

R.A. McKinney, B.H. Gähwiler and S.M. Thompson *Brain Research Institute, Univ. of Zürich, CH-8029 Zürich.*

Seizures are a common consequence of severe head trauma, and axonal reorganization may underlie such lasting changes in excitability. Mature organotypic hippocampal cultures (> 14 days *in vitro*) were used to investigate the capacity of recurrent axon collaterals of CA3 pyramidal cells to sprout in response to transection of the Schaffer collateral pathway with a razor blade. Cultures were allowed to survive for varying times before living CA3 cells were injected with biocytin to observe their axonal arboration, or before processing the cultures for GAP-43 immunohistochemistry, to observe growing axons. Changes in post-synaptic sites were also investigated by injecting living CA1 cells with lucifer yellow and imaging with confocal microscopy. CA1 cells injected at 3 days after transection showed an increase in dendritic spines, whereas there was a dramatic decrease at 7 days. Interestingly, the number of spines returned to normal by 21 days post-transection. Evidence of CA3 cell axonal sprouting was observed as early as 3 days post-lesion. These findings suggest the genesis of new excitatory synapses in injured hippocampus, perhaps resulting in an increase in the relative strength of synaptic excitation versus inhibition, and may provide a model for post-traumatic epilepsy.

S07-25

SYNAPTOGENESIS IN AGGREGATING BRAIN CELL CULTURES IS AFFECTED BY THE MODULATION OF THE NEURONAL ELECTRICAL ACTIVITY

Pardo B., Pithon E. and Honegger P. Institut de Physiologie, Université de Lausanne, 1005 Lausanne.

Synaptogenesis was studied in aggregating fetal rat telencephalon cultures by measuring the developmental expression of cytoskeletal and synaptic proteins. Both total homogenates and synaptosomal fractions of the cultures were analyzed by western blotting. To modulate the neuronal electrical activity, cultures were treated from day 7 to day 20 with depolarizing concentrations of potassium chloride (KCl) or with tetrodotoxin (TTX), a specific blocker of the voltage-dependent Na^+ channel. It was found that treatment with KCl (30 mM) produced a 2-fold increase in the content of neurofilament-H (NF-H) subunit, and of synaptophysin, measured in synaptosomal preparations and in total homogenates. The synapsin content was increased by 40%. Treatment with TTX (2 μM and 10 μM) reduced the total NF-H content by 21 and 50%, respectively. Furthermore, in the synaptosomal fractions, reductions in synaptophysin (-14% and -32%) and in synapsin (-32% and -39%) were found. In contrast, KCl and TTX treatments had little or no effect on NF-M expression. These data support the view that synapse formation is influenced by the neuronal electrical activity, and they validate this three-dimensional cell culture system as a model to study synaptogenesis.

S07-26

The CHEN (chick embryo neural) cell culture assay for neurotoxicology screening

Christoph A. Reinhardt, Dunja M. Frey and Catherine H. Schein

Laboratory for In Vitro Toxicology, Swiss Institute for Alternatives to Animal Testing, SIAT, Technopark, Pfingstweidstrasse 30, CH-8005 Zurich, Switzerland

The CHEN assay uses primary chick embryonal brain and retina cells to determine the neurotoxicological potential of substances. We have found that glutamine synthetase (GS) activity and cell protein are good markers of toxicity (Reinhardt CA & Schein CH, Toxicology in vitro, in the press). Certain chemicals (eg., lindane) increased the specific GS activity of the cultures, suggesting that their toxicity in vivo may be due in part to generation of toxic glutamate. The effects of addition of excitatory amino acids and antagonists of glutamate action were used to further characterize the sensitivity and specificity of the system.
To further optimize the assay, the in vitro development of the different neural cell types in various serum free media were tested in monolayer and reaggregate culture conditions. Other markers tested are changes in the levels of key enzymes and mRNA's for various stress proteins and cytokines. Alterations in the markers throughout the culture period were compared with that occurring in vivo in the developing chick embryo.

S07-27

The blood-brain barrier in vitro: Characterization and potential use for neurotoxicology screening

Nicole Heller & Christoph A. Reinhardt

Laboratory for In Vitro Toxicology, Swiss Institute for Alternatives to Animal Testing, SIAT, Technopark, Pfingstweidstrasse 30, CH-8005 Zurich, Switzerland

Endothelial cells from cortical grey matter of calf brain (purchased from a local slaughterhouse) were prepared and purified by a modification of the procedure of Audus & Borchardt (Pharm. Res. 3:81-87, 1986) using a dextran and a percoll gradient. Fresh and freeze-thawed endothelial cells were cultured on collagen-coated plastic surface and microporous membrane inserts. The purity of the endothelial cell monolayer was monitored morphologically and immunocytochemically. Resistance and permeability (3H-inulin and 14C-glucose) through the endothelial layer was measured in order to determine the integrity of the in vitro blood-brain barrier. The influence of growth factors and astrocyte-conditioned medium on the barrier function of the endothelial layer and the expression of specific proteins (von Willebrand factor, P-glycoprotein, ZO-1, Na,K-ATPase) was tested. This in vitro model for the blood-brain barrier will be investigated in relation to the effect of various neurotoxins.

(Supported by the Stiftung Forschung 3R, Berne and the M. & F. Fleitmann Foundation, Lucerne)

S07-28

INFLAMMATORY REACTIONS AND CYTOKINE EXPRESSION UPON EXPERIMENTAL SPINAL CORD INJURY

Deborah Bartholdi and Martin E. Schwab, Brain Research Institute, University of Zürich, August Forel Str. 1, 8029 CH-Zürich, Switzerland.

Injury of the spinal cord triggers a complex sequence of molecular and cellular reactions which are probably regulated by growth factors and cytokines. We studied by *in situ* hybridization the expression of several cytokines and chemokines (TNF α, Il 6, MIP 1α/β, MIP 2) in the lesioned mouse spinal cord. Interestingly, the specific cytokine pattern at spinal cord lesion sites (early and high expression of MIP 1α /β, undetectable levels of TNF α, Il 6) differs markedly from that observed in a bacterial meningitis model (high levels of MIP 1α/β, TNF α and Il 6). The same types of inflammatory cells are recruited in the trauma and the meningitis model, but the different stimuli may lead to different effector functions of these cells.

S07-29

LYMPHOCYTES AND INFLAMMATORY CELLS IN THE LESIONED SPINAL CORD OF THE MOUSE.

Lisa Schnell, Regula Schneider and M. E. Schwab, Brain Research Institute, University of Zürich, August-Forel-Str. 1, 8029 Zürich, Switzerland.

Primary spinal cord hemisections cause a cascade of cellular reactions which also lead to secondary tissue destruction. A large area of cell death develops within the first hours, affecting neuronal and glial cells. Inflammatory neutrophils infiltrate into the lesion site from 6 hours on, followed by macrophages (Dusart and Schwab, 1994). These inflammatory cells, however, arrive too late to be responsible for the early phase of secondary cell death.

We have observed the appearance of lymphocytes within the lesion area as early as 1 hour after the insult. Although the number of these cells is small compared to neutrophils or macrophages, their continuous increase over the following weeks points towards a specific interaction.

Imunohistochemical analysis of lymphocyte subpopulations revealed the presence of CD4+ and CD8+ T-cells and B-cells at 1-96 hrs. At 2-5 weeks CD8+ lymphocytes are present in clusters even remote from the lesion. Their possible involvement in demyelination remains to be studied.

S07-30

PURIFICATION OF A NEURITE GROWTH INHIBITOR (NI-250) FROM BOVINE CNS MYELIN.

A.A.A. Spillmann, C.E. Bandtlow and M.E. Schwab, Brain Research Institute, University of Zurich, August-Forel-Str. 1, CH-8029 Zurich, Switzerland

Inhibitors of neurite growth are likely to play an important role in the development of the nervous system. Two of them, NI-35 and NI-250, also contribute to the lack of regeneration of CNS axons following lesion. These low abundant glycoproteins which cause collapse of neuronal growth cones and strongly inhibit neurite growth *in vitro* are associated with oligodendrocyte membranes and in the myelin sheath of the central nervous system in higher vertebrates. The strategy for purification starting with crude bovine myelin involves the use of various chromatography steps in combination with three bioassays. By means of anion exchange, reverse phase and size exclusion chromatography, a highly active protein fraction was obtained. The biological activity of this fraction is enriched 1200-fold, is neutralized the monoclonal antibody IN-1 and shows one detectable band at 250kd in SDS-PAGE (silver-stain).

S07-31

ELECTROPHYSIOLOGICAL STUDY OF AXOTOMIZED NEONATAL FACIAL MOTONEURONS OVEREXPRESSING Bcl-2.

Alberi S., Raggenbass M., De Bilbao F. and Dubois-Dauphin M.
Dept. of Physiology, University Medical Center, 1211 Geneva 4.

In wild type (w.t.) two days old mice, lesion of the facial nerve results in rapid degeneration of facial motoneurons. By contrast, in transgenic mice overexpressing the Bcl-2 protein, motoneurons survive axotomy. We have investigated the functional properties of facial motoneurons in w.t. and transgenic mice seven days after unilateral facialectomy, using brainstem slices and whole-cell recordings. Motoneurons from control animals had input resistances of 45 ± 15 MΩ (n=11) and fired repetitively following injection of positive constant current pulses. When cells were voltage-clamped near their resting membrane potential, AMPA or vasopressin generated sustained inward currents. In w.t. axotomized mice, no viable neurons could be recorded ipsilateral to the lesion. By contrast, in transgenic axotomized mice, facial neurons could be found located ipsilaterally to the lesion : they had input resistances of 190 ± 40 MΩ (n=11) fired repetitively and responded to AMPA, NMDA and vasopressin. We suggest that, in transgenic mice, the electrophysiological properties of neonatal facial motoneurons which survive axotomy are, at least in part, similar to those of non-axotomized ones.

S07-32

MICROGLIAL CELL RESPONSE TO OPTIC NERVE CRUSH IN *XENOPUS* TADPOLE

Chantal Alliod and Elisabeth Rungger-Brändle, Microscopie électronique, Clinique d'Ophtalmologie, HCUG, 1211 Genève 14

Successful regeneration of *Xenopus* tadpole optic nerve (ON) heavily relies on an efficient endogenous phagocyting system (microglia and astrocytes) that rapidly removes cell debris and myelin from lesioned tissue. We have studied the dynamics, during the first 10 days postinjury, of microglial cell populations in situ and in explanted ON segments cultured in vitro. Microglia was identified by Isolectin B4 (IB4) binding. Cell counts at the crush site and within the adjacent region of the distal segment revealed differences in microglial cell frequencies with peak values at day 1 and 5 postlesion, respectively. Along the distal segment, their number increased gradually from the lesion site towards far distal but this gradient progressively flattened and had virtually disappeared by day 5 with comparable numbers throughout the whole distal nerve stump. Moreover, total mitotic activity showed marked regional differences.

In explants from normal ON, very few microglial cells were present. However, segments from regenerating ON that had been explanted 1-5 days postlesion, contained numerous microglial cells. Their number depended on the position relative to the lesion site of the explanted segment and closely reflected the in situ distribution. Microglial cells maintained the expression of the IB4 epitope but did not divide once explanted from the lesioned nerve.

S07-33

NEUROTOXIC EFFECTS OF OCHRATOXIN IN VITRO: ESTIMATION OF A NO-EFFECT CONCENTRATION, CELL TYPE- AND TISSUE-SPECIFICITY.

A. Bruinink, C. Sidler and I. Studer
Institute of Toxicology, ETH & Uni Zürich, CH-8603, Schwerzenbach

Ochratoxin A (OTA) toxicity was characterized, using the SCENT concept for neurotoxicity testing (Bruinink et al., Toxicol. Lett. 74 Suppl.1(1994), 10). For this, various concentrations of the compound were added to serum-free primary cultures of embryonic chick neuronal retina, brain and for comparison meninges 24 hours after plating. The threshold OTA concentration was found to correlate with the duration of the treatment period, as concluded from light microscopic examination. The effects on viability and differentiation were determined 7 days after addition of the compound, i.e. the no-effect OTA concentration defined as benchmark concentration 5% (BMC5), EC50 and Nc values of the OTA concentration - effect relationships. Preliminary results suggested that total protein and lysosomal and mitonchondrial activity in the 3 cultures were modified at nearly the same initial OTA concentrations (IC50≈0.3 µM). Nerve cells were affected at slightly lower concentrations (IC50≈0.1 µM) in comparison to glial cells.

In order to determine which part(s) of the OTA molecule is/are important for OTA toxicity, the effects of OTA and ochratoxin B (OTB) were compared, using meningeal cultures. Our results suggested that, nearly 3 times more OTB was needed to achieve the same toxicity in comparison to OTA. In addition, the effects of phenylalanine on OTA toxicity was determined, using brain cell cultures.

S07-34

DIFFERENTIATION OF CHOLINERGIC NEUROBLASTOMA CELLS MIGHT BE MODULATED BY UBIQUITIN EXPRESSION.

Hoang-Van, K., Roulet E**., Eder-Colli, L**. and Jaton, J.-C** ,
*Department of Medical Biochemistry and **Department of Pharmacology, CMU, 1211 Geneva 4

In human, ubiquitin has been shown to accumulate in the cytoplasmic inclusions of several neurodegenerative diseases. It has been reported that in motor neuron disease the generation of ubiquitinated filamentous inclusion bodies formed in neurons resulted in increased transcription of a polyubiquitin gene. Also, transfection in higher plants with polyubiquitin gene induced necrotic lesions. We started experiments aiming to study the function of ubiquitin in a cholinergic cell line; that is, rat neuroblastoma NS20Y. Three parameters were taken into consideration: morphology, choline acetyltransferase (ChAT) and lactate dehydrogenase (LDH) activities. Addition of an anti-ubiquitin antisense oligonucleotide to the culture medium induced an increase of the ratio of ChAT to LDH activity but no morphological change. Transfection of cells with a human polyubiquitin cDNA containing nine repetitive coding units of the C family gene induced no significant alteration of the ChAT/LDH ratio. Transfected cells appeared less differentiated than untransfected ones. Interestinghly, addition of dibutyryl cyclicAMP (dBcAMP) to untransfected cells rapidly induced morphological differentiation (in 2 to 4 hours) with a significant increase of ChAT/LDH ratio. In contrast, preliminary results indicated that these changes did occur with a significant delay (3 to 5 days) in dBcAMP poly-ubi transfected cells.

S07-35

MYELIN OLIGODENDROCYTE GLYCOPROTEIN TRANSLATION IS NOT BLOCKED IN TRANSGENIC MICE EXPRESSING ITS ANTISENSE TRANSGENE mRNA

Jaquet V., Tosic M., Gow A., Lazzarini R. and Matthieu J.-M.Laboratoire de Neurochimie, Service de Pédiatrie, CHUV,Lausanne.

Myelin Oligodendrocyte Glycoprotein (MOG) is a minor myelin specific protein.To understand its function, we made transgenic mice expressing MOG antisense mRNA. Antisense strategy has proved its efficiency in inhibiting gene expression in *in vitro* studies and transgenic plants, but, in animals, there is only one example that have proved successful (Katsuki et al, 1988).We used a DNA construct containing 430 bp of the 5' region of the MOG cDNA in the **reverse orientation** and a strong promoter (MBP).Although Northern blots showed a high expression of the antisense mRNA in transgenics, expression of the endogenous MOG was not inhibited.On the contrary, we detected an up-regulation of the sense MOG gene and of other major myelin genes. Thus the feasibility of antisense strategy *in vivo* remains questionable and the presence of a pleiotropic effect of the transgene is probable.

S07-36

AGE RELATED FIBER ORDER IN THE DEVELOPING OPTIC NERVE OF THE CHICK.

Drenhaus, U., Thomas, K., Rager, G.; Institut für Anatomie und Spezielle Embryologie, Universität CH-1700 Freiburg.

In a quantitative electron microscopic study we analyzed the topographic distribution of axons according to maturity (Drenhaus & Rager, Anat. Rec., 1994) in the optic nerve of the chick embryo (embryonic days 5-7) at three predefined nerve positions.

The majority of growth cones (GCs) is found in the ventral floor of the nerve. Their frequency decreases toward the dorsal region. The distribution of the oldest, i.e. proximal axon segments, is just the opposite. Their number is highest dorsally and decreases toward the ventral region. Distal segments, being of intermediary maturity, present an intermediary topographic position between the former two.

The fiber arrangement indicates a dorso-ventral gradient of maturity. This pattern results from the ventral addition of ingrowing axons. The presence of some GCs further dorsally may be due to the fact that later arriving fibers can follow older ones coming from the same retinal position.

The study was supported by SNF 3100-25663.

S07-37

ULTRASTRUCTURAL ASSOCIATION OF HYALURONAN (HYALURONIC ACID) WITH UNMYELINATED NERVE FIBERS OF RAT SKIN, IRIS AND OPTIC RETINA

Peter S.Eggli and Werner Graber Institute of Anatomy, University of Bern, Switzerland

Neural tissue of central (rat optic retina) and peripheral origin (nerve fascicles of rat iris and skin) was processed by osmium tetroxide/microwave fixation and embedded in epoxy resin. Hyaluronan-binding proteins and link proteins coupled to 15-20 nm gold particles were used as markers in a one-step post-embedding procedure for identifying hyaluronan at the ultrastructural level. The periaxonal space of peripheral unmyelinated nerve fibers and the extracellular space surrounding optic nerve fibers were intensively labelled. The specificity of the hyaluronan-binding probes was demonstrated by the total loss of labelling following treatment of sections with *Streptomyces* or testicular hyaluronidase or by preincubating the probes with hyaluronan oligosaccharides.

S07-38

REGULATION OF NEURON DEATH IN DEVELOPING CNS BY ELECTRICAL ACTIVITY AND TROPHIC FACTORS.

Primi M.-P. and Clarke P.G.H, Institute of Anatomy, University of Lausanne.

Naturally occurring neuronal death is regulated by anterograde and retrograde signals. Both involve electrical activity and trophic factors. We are currently trying to disentangle the roles of activity and trophic factors in ***retrograde*** signalling to the isthmo-optic nucleus (ION: source of efferents to the retina) in chick embryos.

Intraocular injections of the neurotrophin BDNF, or of saxitoxin, both reduce neuronal death in the ION. The survival promotion by BDNF is due, at least partly, to a direct action on the isthmo-optic axons; it occurs even when the target cells have been destroyed with kainate.

The retrograde influence of saxitoxin is likewise due, at least partly, to a direct, presynaptic action. It is too fast to be due to a modulation of the synthesis or release of BDNF or other trophic factors, occurring within 6 hours. We think action potentials may lead to a chain of second messenger events in the axon terminal that is ultimately signalled retrogradely through the transport of a long-lived second messenger. Such signals may complement conventional ones involving the transport of neurotrophin-receptor complexes.

S07-39

VISUALIZATION OF DIFFERENT COW RETINAL CELL TYPES BY IMMUNOHISTOCHEMISTRY

DE RAAD S. and SCHORDERET M., Dept. of PHARMACOLOGY, C.M.U.

This communication relates the use of cell-specific monoclonal and polyclonal antibodies to specifically label cell types of the cow retina. The following antibodies stain:

- Anti-GFAP — the Müller cells and the astrocytes.
- Anti-recoverin — the photoreceptors (PRs) and two types of bipolar cells (BCs).
- RET-P1 — the PRs and some BCs.
- Anti-CaBP-28 — the horizontal cells (HCs), some amacrine cells (ACs) and some ganglion cells (GCs).
- 8A1 — HC ramifications, rare ACs and the GCs.
- HPC-1 — the ACs.
- AB5 — the GCs.

The development of new cell-specific monoclonal antibodies and their use as neuroanatomical probes was shown to be a very powerful tool for deciphering the structural and functional organization of the retina.

S07-40

ORGANISATION OF THE WHISKER REPRESENTATION IN THE SOMATOSENSORY CORTEX (SI) OF THE MOUSE MUTANT *BARRELLESS*; A DEOXYGLUCOSE STUDY.

Egbert Welker & Gilles Bronchti, Institute of Anatomy, University of Lausanne, 1005 Lausanne.

In SI of mice the representation of an individual mystacial vibrissa has a morphological correlate in the form of a barrel in layer IV. *Barrelless* mice lack barrels in SI, but have whisker-related patterns in the lower stations of the pathway. Here, we investigated the cortical whisker representation in *barrelless* (n=8) and NOR-mice (n=6). They had all whiskers on the left side clipped except those of follicles C1-3 (group I), or of follicles B1-3 and D1-3 (group II). After the injection of ^{14}C-deoxyglucose mice explored an object-filled cage. Then they were anaesthetised, transcardially perfused and their hemispheres cut tangential to the pial surface above SI. Analysis of DG uptake patterns showed that the cortical whisker representation in *barrelless* is topologically organised in an identical manner as in NOR. A quantitative analysis revealed that areas of stimulus-dependent DG uptake were larger in *barrelless* than in NOR-mice. We conclude that in *barrelless* the whisker representation in SI is somatotopically organised and suggest that the larger representation of individual whiskers is due to a diminished spatial segregation of the thalamo-cortical afferents in layer IV. Support: Swiss NSF 31-39184.93

S07-41

MOLECULAR MECHANISMS OF NEURONAL SELECTION DURING DEVELOPMENT. Catsicas, S., Di Paolo, G., Staple, J. and Grenningloh, G. Glaxo Institute for Molecular Biology, Geneva, Switzerland.

To identify genes involved in the process of neuronal selection, we used differential DNA-DNA competitive hybridization during specific developmental stages. We have cloned 55 partial cDNAs of genes expressed in the chick retina during synapse formation and developmental cell death. One of the subtracted clones encoded the phosphoprotein stathmin whose expression was maximal at the stage of neuronal selection. Specific inhibition of stathmin expression in PC12 cells with antisense oligonucleotides resulted in their inability to respond to NGF. The cells continued to proliferate and did not extend processes. PC12 cells can also be used to study neuronal death. In serum-free medium, terminally differentiated PC12 cells die by apoptosis, which can be prevented by NGF. We found that stathmin antisense treated PC12 cells could not be rescued by NGF in serum-free medium. Altogether, our results demonstrate that stathmin is a critical component of the NGF signaling pathway.

Diabetes susceptibility genes

S08-01

EFFECTS OF LIGANDS AT 5-HT RECEPTOR SUBTYPES IN A PALATABLE FOOD CONSUMPTION PARADIGM IN THE RAT

J.R. Martin, J.-L. Moreau and F. Jenck. Pharma Division, Preclinical Res. Dept., Bldg. 72/150, F. Hoffmann-La Roche Ltd, CH-4002 Basel

Compounds acting selectively, or predominantly, at the different 5-HT receptors exhibit different effects on food intake. In this study rats were exposed to palatable food (boiled potatoes) until intake stabilized. Test compounds were given orally 30 min prior to a 30-min evaluation of food intake. Days on which drug or vehicle treatment was given alternated with days without. A repeated-measures design was used with a minimum of 48 h separating test days. 5-HT1A receptor agonists and partial agonists enhanced intake (active doses are shown; mg/kg): 8-OH-DPAT (1-10), buspirone (0.3-10), gepirone (30), ipsapirone (1-10), flesinoxan (1-3); whereas the antagonist MDL73005EF (0.3-10) given alone was inactive. In contrast, nonselective 5-HT2C receptor agonists reduced intake: mCPP (0.3-3), DOI (3), ritanserin (0.3), MK212 (1-10), and TFMPP (1-3); whereas the antagonist SB200464A (10-30) given alone was inactive. In conclusion, 5-HT1A receptor agonists enhanced intake but an antagonist was inactive, whereas 5-HT2C receptor agonists reduced intake but an antagonist was inactive. This pattern of results obtained in this palatable food paradigm is consistent with that reported for 5-HT1A and 5-HT2C receptor ligands in other test situations and, furthermore, suggest the possible value of 5-HT2C receptor agonists in the therapy of eating disorders.

S08-02

IGF-1 IN THE AVIAN AND REPTILIAN PANCREAS

Reinecke M.[1], Brun R.[1], Broger I.[1], Zapf, J.[2] and Maake C[1].
Institute of Anatomy[1] and Department of Internal Medicine[2], University of Zürich, Zürich.

The presence of IGF-1 in the endocrine pancreas of reptiles and birds was studied using antisera against IGF-1 and the classical islet hormones insulin (INS), glucagon (GLUC), somatostatin (SOM) and pancreatic polypeptide (PP) in double immunofluorescence. In all species studied, IGF-1-immunoreactivity (-IR) was observed in islet cells. In birds, IGF-1-IR occurred either in SOM- (*Gallus g. domesticus, Streptopelia roseogrisea*) or in PP-immunoreactive (*Coturnix c. japonica*) cells. In reptiles, the lizard species (*Scincus officinalis, Lacerta viridis*) exhibited IGF-1-IR in PP-immunoreactive cells, and the snake species *Psamophis leniolatum* and *Coluber ravergieri* in GLUC-immunoreactive cells. In none of the species studied, IGF-1-IR was present in INS-immunoreactive cells. Thus, the distribution patterns of IGF-1-IR in reptiles and birds are equivalent to those obtained before in mammals, amphibia and most bony fish. They differ, however, from the localisation found in lower vertebrates and protochordates where a total or partial co-existence of IGF-1- and INS-IR was obtained. Summarising it can be stated, 1.) that the existence of IGF-1-IR may be common in the islets of all vertebrates, and 2.) that the divergence of IGF-1 and INS seems to have occurred at the phylogenetic level of lower vertebrates.
Supported by the Swiss National Foundation (grant 32-33349.92).

S08-03

EXPRESSION OF IGF-1 IN THE GASTRO-ENTERO-PANCREATIC SYSTEM OF BONY FISH

Cueni D., Graf H., Hasler R. and Reinecke M.
Institute of Anatomy, University of Zürich, CH-8057 Zürich

Immunohistochemical studies on the phylogeny of IGF-1 revealed that in 5 out of 6 bony fish species studied, IGF-1-immunoreactivity occurred in somatostatin, glucagon or PP cells, but no IGF-1-immunoreactivity was found in insulin (INS) cells. As exception, *Cottus scorpius* showed 100% coexistence of INS- and IGF-1-immunoreactivites. In order to study the site of synthesis of IGF-1 in bony fish, molecularbiological techniques were applied. Total RNA from liver, pancreas and gut of trout, carp and *Cottus scorpius* was extracted and cDNA was synthesized. For PCR, primers flanking the C-, A-, and D-domain of trout IGF-1 were designed. Hybridization was carried out using a PCR product of trout liver (242 bp) as hybridizationprobe. The specificity of the probe was verified by restriction digestion. Trout PCR revealed 2 typical bands at 242 bp and about 320 bp. In *Cottus*, the specific bands were slightly shorter, respectively, longer. The PCR products of *Cottus* and carp were analysed and compared with those of trout. Summarising it can be stated, that IGF-1 is expressed not only in teleost liver, but also in pancreas and intestine. Furthermore, different forms of IGF-1 seem to be expressed among teleosts.
Supported by the Swiss National Foundation (grant 32-33349.92) and the Foundation for Scientific Research (University of Zürich).

S08-04

ADAPTATION OF MEAN INSPIRATORY FLOW TO BRONCHO-CONSTRICTION IN INSULIN-DEPENDENT DIABETICS WITH AND WITHOUT AUTONOMIC NEUROPATHY

Voser P., Savoy J., Tempini A., Haab P., Physiology Dept. University of Fribourg, CH 1700, Switzerland

In subjects with insulin-dependent diabetes mellitus **(IDDM)**, diabetic autonomic neuropathy **(DAN)** was shown to alter neither the initial inspiratory drive **(P0.1)** nor its adaptation to bronchoconstriction. The present study investigates, whether the mean inspiratory flow **(V_T/T_i)**, which is an expression of the mean inspiratory drive, is altered in DAN. We report data obtained on the same subjects as above. In two groups of subjects with IDDM (one group with (n=12) and the other one (n=9) without DAN) V_T/T_i was calculated before and after a Methylcholine-inhalation **(M)** inducing in both groups a mean fall of FEV1 by 19%. DAN was scaled from 0-8 **(S)** according to multiple standard tests.
Results: Mean values ±SEM; VT/Ti in l (BTPS)/s; *: p<0.05

Groups	S	V_T/T_i control		V_T/T_i after M
DAN (+)	4.92±0.47	0.40±0.02	*	0.52±0.04
DAN (-)	1.22±0.28*	0.42±0.02	*	0.52±0.03

Under bronchoconstriction VT/Ti increases in both groups to the same extent. This observation suggests, that V_T/T_i is not altered in diabetic autonomic neuropathy. Supported by ASFC.

S08-05

EFFECTS OF GLUCAGON ON FRUCTOSE-INDUCED ALTERATIONS OF GLUCOSE METABOLISM IN MAN.

Tappy L., Schneiter Ph., Paquot N., Jéquier E., Institut de Physiologie de l'Université, Bugnon 7, 1005 Lausanne.

Gluconeogenic substrates increase gluconeogenesis but fail to enhance overall endogenous glucose production (EGP). In order to assess the effects of hyperglucagonemia on EGP, 8 healthy subjects were studied a) during hourly ^{13}C fructose ingestion (0.3 g/kg fat free mass/hour) for 3 hours (F); b) during ^{13}C fructose ingestion + hyperglucagonemia (232±9 ng/L) (F+G); c) during hyperglucagonemia alone (G). EGP was assessed with 6,6 ^2H glucose and fructose gluconeogenesis from ^{13}C plasma glucose. EGP increased by 22 % with F+G (p<0.05) but remained unchanged during F or G. Plasma ^{13}C glucose was identical with F and F+G, indicating a similar relative contribution of fructose gluconeogenesis to the glucose-6-phosphate pool. It is concluded that a) both an increased glucagonemia and an enhanced supply of gluconeogenic precursors are required to increase EGP; b) F+G increase EGP without altering the relative proportion of glucose-6-phosphate production from fructose and from other sources (i.e. glycogenolysis + gluconeogenesis from non fructose precursors). This suggests that alterations of glycogen synthesis or of glucose-6-phosphate hydrolysis are involved.

S08-06

DO THE PINEAL GLAND AND MELATONIN PLAY A ROLE IN DIABETES MELLITUS TYPE I ?

Ario Conti and Georges J.M. Maestroni
Centre for Experimental Pathology, Istituto Cantonale di Patologia, Locarno, Switzerland, 6604.

We have demonstrated that the pineal gland and melatonin (MLT) play an immunoregulatory role both in mice and in humans. In particular: **a)** MLT enhances the primary antibody response in normal and stressed animals; **b)** counteracts the immunosuppressive effect induced by acute stress and/or by corticosteroid treatment and **c)** exerts these interesting effects triggering the synthesis and/or release of opioid peptides from activated CD4+ ,T lymphocytes. Furthermore MLT acts of T helper lymphocytes inducing the release of IL 4. These findings prompted us to investigate the role of the pineal gland and MLT in diabetes mellitus type I. The NOD mouse has been used as experimental model. Preliminary results shows that in female NOD mouse: **1)** neonatal pinealectomy accelerate the development of the disease; **2)** exogenous MLT increase the production of IAA as expected.
Further studies are in progress in our laboratory to assess the role of the pineal gland and melatonin in the immunopathogenesis of diabetes mellitus.

S08-07

CLONING, FUNCTIONAL EXPRESSION AND CHROMOSOMAL LOCALIZATION OF THE HUMAN PANCREATIC ISLET GLUCOSE-DEPENDENT INSULINOTROPIC POLYPEPTIDE (GIP) RECEPTOR

S. Gremlich[1], A. Porret[1], H. Hani[2], D. Cherif[2], N. Vionnet[2], D. Cohen[2], P. Froguel[2], B. Thorens[1]

[1] Institut de Pharmacologie et Toxicologie, Université de Lausanne (CH); [2] Centre d'Etude du Polymorphisme Humain, Paris (F).

GIP is a hormone secreted by the endocrine K cells from the duodenum which stimulates glucose-induced insulin secretion. The human pancreatic islet GIP receptor was cloned from a pancreatic islet cDNA library. Two different spliced forms were found, which differ by the insertion of 27 amino acids in the carboxy-terminal tail. Both forms of the receptor were stably expressed in Chinese hamster lung fibroblasts. They displayed high affinity binding for GIP (180 and 600 pM). Glucagon, GLP-1(7-36)amide, VIP and secretin, did not significantly displace GIP binding at 1 µM. However, two related peptides, exendin-4 and exendin-(9-39) displaced GIP binding by almost 70% at 1 µM. GIP binding to both forms of the receptor dose-dependently increased intracellular cAMP levels (EC50s of 0.6-0.8 nM). No increase in intracellular calcium could be recorded following GIP binding, suggesting that the receptor is mainly coupled to activation of adenylyl cyclase. Furthermore, we showed that both exendin-4 and exendin-(9-39) were antagonists of the receptor, inhibiting GIP-induced cAMP formation. The physical and genetic chromosomal localization of the receptor gene was determined to be on 19q13.3 close to the ApoC2 gene.

S08-08

IN VIVO REGULATION OF GLUT2 IN TRANSPLANTED β-CELL

Roduit R*, Waeber G# and Thorens B*.

*Inst. Pharmacology and Toxicology, Lausanne, # CHUV, Lausanne.

Expression of the pancreatic β cell glucose transporter GLUT2 is decreased in glucose unresponsive islets of diabetic mice and rats. To study the molecular basis for this regulation we have developed a transplantation technique to expose pancreatic islets or insulinoma cells to the environment of rodent models of diabetes. Islets are encapsulated in semi-permeable PAN-PVC capsules and implanted intraperitoneally into host animals. The capsules are retrieved after different periods of time and the expression of GLUT2 is analysed by immunohistochemistry and by Western blot analysis. Ten days after transplantation in control rats, the encapsulated islets expressed normal Glut2 protein level. In contrast, when transplanted in streptozocin-diabetic (STZ) rats GLUT2 expression was strongly reduced. To test the role of high glucose on GLUT2 expression, the glycemia was corected by phlorizin treatement. This treatement led to a reexpression of GLUT2 to normal level. However, exposure of islets to high glucose concentrations *in vitro* led to increase GLUT2 expression. Thus the direct effect of glucose on GLUT2 expression is a stimulatory one. Therefore, in diabetes, hyperglycemia probably induces secondary factors which have a dominant negative effect on GLUT2 expression. To try to determine which region of the GLUT2 promotor are implicated in this regulated expression, we are applying this transplantation technology to the insulinoma cell line INS-1. These cells express endogenous GLUT2 and have further been transfected with a GLUT2 promotor-CAT construct. After encapsulation, transplantation and retrieval, the CAT activity can be measured. Using this approach we hope to be able to determine the promotor region involved in transporter regulation in diabetes.

S08-09

RECOMBINANT HUMAN INSULIN ANALOGUES LABELLED WITH STABLE ISOTOPES AND THEIR USE FOR IN VIVO PHARMACOKINETIC STUDIES BY MASS SPECTROMETRY

Stöcklin, R., Arrighi, J.-F., Hoang-Van, K., Vu, L., Cerini, F., Rose, K. & Offord, R.E. Département de Biochimie Médicale, C.M.U., 1211 Geneva 4, Switzerland.

In order to study the pharmacokinetics of proteins without introducing radioactivity into the body, we propose a new method, based on isotope dilution mass spectrometry, and exemplify it using insulin derivatives labelled with stable isotopes. We have developed a bacterial expression system that allows production of uniformly labelled analogues of proinsulin in fusion with a poly-histidine tag. The use of specific culture media enriched with stable isotopes allows us to produce uniformly labelled analogues of this precursor. After purification, refolding and transformation steps, we obtain proinsulin, insulin and C-peptide enriched for example with 99.4% ^{15}N, 25% deuterium, or 99.987% ^{12}C (negative labelling, ^{13}C depleted). Such derivatives of different molecular weight are authentic, non radioactive and can be distinguished from their endogenous form by electrospray MS analysis.

S08-10

Functional and structural identification of the *cis*-elements and *trans*-acting factors of the high Km Glucose Transporter GLUT2 promoter controlling the pancreatic ß-cell specific expression of the gene.

N.Thompson, C.Bonny, P.Nicod and G.Waeber.

Department of Internal Medicine B, CHUV-1011 Lausanne,Switzerland

In glucose-unresponsive ß cells of experimental models of type II diabetes the GLUT2 glucose transporter isoform expression has been found to be specifically suppressed. In order to identify the *cis*-elements and *trans*-acting factors involved in the abnormal ß-cell specific expression of GLUT2, we have cloned the upstream regulatory region of the gene. Transgenic mice were generated that have integrated -1311 to + 49 bp of our identified GLUT2 promoter linked to the chloramphenicol acetyl transferase (CAT) reporter gene. The transgene was expressed in the Langerhans islets of the generated transgenic mice. Deletion constructs of our defined promoter were then tested by transient transfection experiments in ß and non-ß cell lines (INS-1, ßTC-3,1027-B2, InRG9, JEG-3). The -1311 to -338 bp of the GLUT2 promoter contain repressor elements whereas the proximal region shows high CAT activity. Mobility shift assays and DNA footprinting experiments with the proximal region of the GLUT2 promoter (-338 to +51 bp) have defined 3 specific *cis*-elements that interact with ubiquitous and ß-cell specific *trans*-acting factors. Various combinations of the 3 *cis*-elements have been cloned upstream of the minimal thymidine kinase promoter (TKCAT) and by transient transfection we show specific activities associated with these elements. As the nucleic acid sequence of these newly defined *cis*-elements are highly conserved within human and mouse,they may have a functional importance in understanding the early events involved in the onset of type II diabetes.

Scanning probe microscopes in biology

S09-01

PROTEIN IMMOBILISATION FOR SCANNING FORCE MICROSCOPY

E. DROZ, M. TABORELLI, T.N.C. WELLS, P. DESCOUTS

The adhesion of biomolecules on the substrate is a necessary condition to obtain reliable images by scanning force microscopy in air or in water. Here, we report different immobilisation procedures on gold surfaces adapted to protein molecules (immunoglobulin native or domains, albumin). These include either the surface functionalisation or the protein modification. Both investigations take advantage of the spontaneous reaction between gold and sulfur atoms (covalent bond). In the first case, thioalcanes with charged groups are grafted on the gold surface in order to increase the surface hydrophilicity and promote an homogeneous distribution of proteins. Covalent binding to the protein can also be performed thanks to coupling reagent. In the second case, sulphydryl groups are introduced on the protein (thiolation). This procedure enables to observe discrete, individual proteins covalently bound on the gold surface. Reproducible images can then be obtained in air as well in liquid. The dimensions of the proteins depend on the applied force on the sample and the geometry of the tip.

S09-02

SCANNING FORCE MICROSCOPY OF DNA AND ITS SUPERCOILING

B. Samori, University of Cosenza and University of Bologna, Italy

An outline of the most significant progresses in Scanning Force Microscopy (SFM) imaging of nucleic acids and nucleoprotein assemblies will be given, emphasizing the most important biological and structural results obtained by this technique in this field.

SFM has been applied to the study of plectonemic supercoiling in DNA plasmids under torsional stress. It was shown that with the aid of this microscopy the topology of the individual supercoiled molecules can be completely characterized.

Chromatin has been also studied by SFM and important insights into its structure have been obtained.

S09-03

CHARACTERIZATION OF PHOSPHOLIPID LANGMUIR BLODGETT FILMS AND PROTEINS BY AFM, XPS AND TOF-SSIMS .

J.M. SOLLETTI (1), M. BOTREAU (2), F. SOMMER (2-3), W.L. BRUNAT (2-3), TRAN MINH DUC (2), M.R. CELIO (1).

(1) Institute of Histology, University of Fribourg, Pérolles, CH-1700 Fribourg, Switzerland.
(2) CENATS, University Claude Bernard, 43 Bd. du 11 Novembre 1918, F-69622 Villeurbanne Cedex, France.
(3) BIOPHY RESEARCH, Novacité alpha, 43 Bd. du 11 Novembre 1918, BP 2131, F-69603 Villeurbanne, France.

This study concerns the elaboration and characterization of DPPE - (1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine) and DPPC - (1,2 - dihexadecanoyl-sn-glycero-3-phosphocholine) and mixed DPPE / DPPC Langmuir-Blodgett (LB) films. We also characterize the proteins (calmodulin) and the plasma membrane Ca-ATPase. AFM was used in order to characterize the surface topography of the deposit at various pressures and at different stochiometric ratios of DPPE/DPPC. Nanometric resolution were obtained on DPPE crystal and DPPE LB films. In the same conditions of deposition, DPPC formed a mixed structure of flat domains and liposomes. At a stochiometric ratio of 50% DPPE, 50% DPPC we observed a better coverage of the substrate with the bilayer. Mass spectra of DPPE- ,DPPC- and DPPE-DPPC LB films were obtained by ToF-SSIMS analysis. The phospholipids were characterized both by their molecular ions and significant fragment ions in positive and negative detection mode. Structural information about the deposit was obtained. The chemical composition and the mean thickness for DPPE and DPPC deposit were also estimated using XPS analysis. Both calmodulin and Ca-ATPase were analysed with XPS and with ToF-SSIMS. Our next aim is to include the proteins in the LB films and to characterize them with this combinaison of methods.

S09-04

Biological samples observed with a thermoregulated AFM.

Jean-Marie Solletti, Raffaella Cargnello, Sandor Kasas †, Franz Haas* and M.R. Celio.

Institute of Histology, University of Fribourg, Pérolles, CH-1705, Switzerland.

* IC Interconnex AG. Hardstrasse 10, CH-5600 Lenzburg, Switzerland.

† Tokyo Institute of Technology, Faculty of Biosciences, 4259 Nagatsuta, Midoriku, Yokohama 227, Japan.

Biological processes depend on many parameters, one of the most important being the temperature. Temperature influences the dynamics of biochemical reactions and the shape of macromolecules. In order to investigate the role of temperature upon some biological samples (cells, bacteria, liposomes and Langmuir-Blodgett films), we built a thermoregulated chamber. In this chamber, we inserted the Nanoscope II / III AFM, in which we can set a given temperature between 0 and 60 degrees. On this poster we report preliminary observations made with this system. We obtained nanometric resolution on mica at different temperatures. In order to compare the fluidity of plasma membranes, we observed different types of bacteria and cells at different temperatures.

S09-05

A general method for immobilizing proteins on an ultraflat substrate for scanning probe microscopy in aqueous bufffers: SFM of clathrin cages and its in-situ disassembly to triskelia on Au-supported monolayers of dithiobis-(succinimidylundecanoate)(DSU).

P. Wagner*, M. Hegner*°, P. Kernen*, E. Ungewickell#, H.-J. Güntherodt°, and G. Semenza*
*Department of Biochemistry, ETH-Zentrum, CH-8092 Zurich, Switzerland,
#Department of Pathology, Washington University, St. Louis, MO 63110
° Institute of Physics, University of Basle, CH-4056 Basle.

Scanning probe microscopy (SPM) has proven its great value in materials science and solid state physics. Its use on biological objects still lags behind, due to their particular properties and the difficulties involved. However, SPM has the potentialities of eventually allowing ultrastructural investigations of native biomolecules under "physiological" conditions, i.e., native, in the presence of ligands and/or effectors, etc. A potentially promising substrate onto which to anchor biomolecules under mild conditions is gold. We have recently succeeded to prepare ultraflat, very large Au(111) surfaces for both STM and SFM [1]. Biomolecules can be chemisorbed via thiolate bonds either (i) by introducing (extra) thiol groups into the biomolecule (e.g. DNA [2], proteins, or lipid vesicles) or (ii) by forming ω-functionalized self-assembled monolayers (SAM), providing a highly reactive "carpet". A new long-chain SAM of DSU has now been prepared on our ultraflat Au surfaces. It binds proteins covalently in aqueous buffers. We present here an SFM study of clathrin cages and the in-situ disassembly to triskelia by using the DSU-SAM for immobilization [3]. Other proteins have also successfully imaged.

[1] Hegner, M.; Wagner, P.; Semenza, G. *Surf. Sci.* **1993**, 291, 39.
[2] Hegner et al. *FEBS Lett.* **1993**, 336, 452.
[3] Wagner et al. *FEBS Lett.* **1994**, 356, in press.

S09-06

Scanning Probe Microscopy - Molecular Measurements and Instrumentation

H. Kumar Wickramasinghe and Frederic Zenhausern, IBM T.J. Watson Research Center, P.O. Box 218, Yorktown Heights, Ny 10598, USA

The current status of techniques and applications of scanning probe microscopy will be discussed. The last decade has seen a significant increase in the utility of both tunneling and force microscopy in academic and industrial laboratories. Applications to organic systems, semiconductors and process measurements are now possible since various types of interactions between the probe tip and sample have been investigated thereby providing achievable resolution up to the atomic level. With the use of new technology, it is possible to image other physical properties of a surface than the density of electronic states provided by the Scanning Tunneling Microscope.

Among these microscopes also relying on a scanning probe is the Atomic Force Microscope (AFM) which is based on controlling the repulsive atomic force between a tip and even insulating sample. We developed a non-contact version of the AFM which uses a laser probe to detect the modulation in mechanical resonance of a cantilever force sensor which is now capable of measuring forces down to 10^{-16} N and force gradients down to 10^{-6} N/m. This extremely high force sensitivity allows one to probe other physical and chemical properties of surfaces. The magnetic force microscope (MFM) and its derivatives have for example allowed one to do local scale NMR imaging. The electrostatic force microscope allows one to measure charge distributions and electric potential on the nanoscale. Other microscopes that have evolved out of our work are the thermal microscope, the Kelvin probe force microscope and the apertureless near-field optical microscope. The operating principles and representative results from these various forms of nanoscale microscopies will be reviewed and results from various labs on applications to biological and molecular measurements will be presented.

S09-07

Time lapse imaging of biological samples using the atomic force microscope (AFM).

S.Kasas, R. Cargnello*, J.M. Solletti* and A. Ikai
Tokyo Institute of Technology, Faculty of Biosciences, 4259 Nagatsuta, Midoriku, Yokohama 227, Japan and
*Institut d'Histologie et d'Embryologie Générale, Pérolles, CH-1700 Fribourg, Switzerland

The AFM developed by Binnig and co-workers belongs to the new, rapidly growing family of scanning probe microscopes. The AFM allows the observation of conducting and non-conducting samples with high lateral and vertical resolution in several mediums such as vacuum, air and different fluids. This possibility makes this instrument very interesting in various biological applications. The AFM allows also the observation of living samples in their natural environment and permits to follow dynamic processes affecting biological material with a spatial resolution of several nanometers. It is this characteristic of the instrument which has been exploited in the present study. We will show some examples of dynamic phenomena affecting living organisms. Several video sequences of motion at the surface of living cells such as yeast and bacteria will be presented and discussed. We believe that this type of time-lapse imaging is going to be more and more used by the AFM microscopist to study the dynamics of biological material and its behaviour when exposed to different chemicals.

Membrane biogenesis

S10-01

YEAST GLYCOSYLPHOSPHATIDYLINOSITOL ANCHORS BIOSYNTHESIS

Benghezal M., Lipke P.N.+, and Conzelmann A.. Institute of Biochemistry, University of Fribourg, Pérolles, CH-1700 Fribourg, Switzerland.
+ Departement of Biological Sciences, Hunter College of City, University of New York, New York NY 10021, USA.

Glycosylphosphatidylinositol (GPI) anchored membrane proteins are synthesized by the posttranslational attachement of a preformed glycolipid to newly made glycoproteins. α-Agglutinin is a GPI-anchored glycoprotein which gets expressed at the cell surface of MATα cells after induction with type a mating factor. Mutants deficient in the biosynthesis of GPI anchors were obtained by selecting for the absence of α-agglutinin from the cell wall after induction with a factor at 37°C. 10 recessive mutants were grouped into 6 complementation classes, gpi4 to gpi9. Although most of the mutants still incorporate significant amounts of *myo*-[2-^{3}H]inositol into GPI anchored proteins at 37°C, they are considered to be deficient in the biosynthesis of GPI anchors since they accumulate abnormal, incomplete GPI glycolipids containing 0, 2 or 4 mannoses. One further mutant accumulates a complete precursor glycolipid suggesting that it is deficient in the transfer of complete precursor lipids to proteins. Invertase and acid phosphatase are normally secreted by all mutants. All mutants show an increased sensitivity to calcofluor white and hygromycin B. This suggests that GPI anchored proteins are required for the integrity of the yeast cell wall.

S10-02

Peroxisome biogenesis and function in *Saccharomyces cerevisiae* targeting of proteins and transport of metabolites

Henk F. Tabak
E.C. Slater Institute, Department of Biochemistry,
AMC, Meibergdreef 15, 1105 Amsterdam, The Netherlands

Peroxisomes are essential subcellular structures of eukaryotic cells with a specific complement of enzymes participating in cellular metabolism. The price paid for the advantage of a separate organelle is that its maintenance requires a set of additional components to translocate proteins and metabolites across the peroxisomal membrane. To search for proteins involved in these processes we developed genetic tests to isolate yeast mutants compromised in such functions. This approach has led to the identification of genes coding for proteins involved in peroxisome function and biogenesis. Their role will be discussed.

S10-03

RETENTION IN THE ENDOPLASMIC RETICULUM (ER) :

ROLE OF COAT PROTEINS

Letourneur, F., Gaynor, E.C., Hennecke, S., Démollière, C., Duden, R., Emr , S.D., Riezman, H. and Cosson, P.

Basel Institute for Immunology, Grenzacherstrasse 487, Postfach CH-4005, Basel, Switzerland

Dilysine motifs in cytoplasmic domains of transmembrane proteins are signals for their continuous retrieval from the Golgi back to the ER. We describe a system to assess retrieval to the ER in yeast cells. Whereas retrieval was unaffected in most mutants tested, a defect in retrieval was observed in previously characterized coatomer mutants, as well as in newly isolated retrieval mutants (*sec21-2*, *ret1-1*). *RET1* was cloned by complementation and found to encode the α subunit of coatomer. Coatomer from β'-COP and α-COP (*ret1-1*) mutants, but not from γ-COP mutants, had lost the ability to bind dilysine motifs *in vitro*. These results suggest that coatomer plays an essential role in retrograde Golgi-to-ER transport and retrieval of dilysine-tagged proteins back to the ER.

S10-04

YEAST GAA1p IS REQUIRED FOR ATTACHMENT OF A COMPLETED GPI ANACHOR ONTO PROTEINS

Dirk Hamburger, Mark Egerton and Howard Riezman, Biozentrum of the University of Basel, 4056-Basel

Anchoring of proteins to membranes by glycosylphosphatidyinositols (GPI) is ubiquitous among all eucaryotes and heavily used by parasitic protozoa. GPI is synthesized and transfered *en bloc* to form GPI-anchored proteins. The key enzyme in this process is a putative GPI:protein transamidase that would cleave a peptide bond near the carboxyterminus of the protein and attach the GPI by an amide linkage. We have identified a gene, *GAA1*, encoding an essential ER protein that is required for GPI anchoring. *gaa1* mutant cells synthesize the complete GPI anchor precursor at non-permissive temperature, but do not attach it to proteins. Overexpression of *GAA1* improves the ability of cells to attach anchors to a GPI-anchored protein with a mutant anchor attachment site. Therefore, Gaa1p is required for a terminal step of GPI anchor attachment and could be part of the putative GPI:protein transamidase.

S10-05

N-LINKED GLYCOSYLATION OF PROTEINS IN THE ENDOPLASMIC RETICULUM OF *SACCHAROMYCES CEREVISIAE*

Aebi,M., te Heesen,S., Stagljar,I., Zufferey, R., Reiss,G. and Burda,P. Mikrobiologisches Institut, ETH Zürich

N-linked glycosylation of proteins is a highly conserved process in the endoplasmic reticulum of eukaryotic cells. The core oligosaccharide $Glc_3Man_9GlcNAc_2$ is transferred from the lipid dolichol phosphate to selected asparagine residues of nascent polypeptide chains, a reaction catalysed by the N-oligosaccharyl transferase complex.
Using yeast genetic techniques, we have identified different components of this multimeric enzyme. To study the complex biosynthetic pathway of the lipid-linked core oligosaccharide, we have isolated different loci encoding enzymes necessary for the synthesis of the core oligosaccharide. The cloning of these genes will make it possible to clarify the topology of oligosaccharide biosynthesis at the ER membrane.

S10-06

PARTITION STUDIES IN LIPOSOME/BUFFER SYSTEMS

Krämer St.[1], Pauletti G.M.[2] and Wunderli-Allenspach H.[1]
[1]Department of Pharmacy ETH, CH-8057 Zürich; [2]Department of Pharmaceutical Chemistry, University of Kansas, Lawrence KS66045-2504 USA.

The pH-dependent partitioning of ^{3}H-[*RS*]-propranolol is studied between unilamellar liposomes consisting of various lipids and a universal buffer with constant ionic strength adjusted to physiological osmolality. The apparent partition coefficient (APC) determined with the phosphatidylcholine (PhC) liposome/buffer system follows the ionisation of both the drug (pK_a 9.24) and the PhC (pK_a 2). The APC at values between 4 and 6 equals 574, the true partition coefficient is 1879. For the more complex systems like PhC/phosphatidylethanolamine(PhE) (7/3 w/w), PhC/phosphatidylserine/-inositol (85.5/8.6/5.9) liposomes and MDCKsomes, i.e. liposomes consisting of lipids from the Madin Darby canine kidney cell line (PhC/PhE/cholesterol/triglycerides/-others 43/31/17/6.8/-2) we found a significantly different partition behavior. The APC in the MDCKsome/buffer system increases from 850 at pH 7 to 1700 at pH 9.5. Above pH 9.5 it decreases to 1300 (pH 11). The other systems show similar results. Zetapotential measurements over the examined pH range reveal a neutral surface charge for PhC liposomes and a negative one for MDCK-somes. Further investigations will focus on the partitioning of other solutes and the influence of various lipids and buffer compounds.

S10-07

TRANSPORT FROM TGN IS CONTROLLED BY PHOSPHORYLATION

Dumermuth E. and Moore H.-P. H., U.C. Berkeley, USA

Endocrine cells possess two different mechanisms for protein transport which diverge at the site of the *trans*-Golgi network (TGN), a constitutive and a regulated secretory pathway. The molecular basis of secretory vesicle formation, as well as the assembly and maturation of regulated granules is only poorly understood. We have developed an *in vitro* system which reconstitutes TGN to cell surface transport of pro-opiomelanocortin hormone (POMC) in SL-O-permeabilized AtT-20 cells. POMC is sulfated in the TGN and is cleaved to mature ACTH in the granule. A significant fraction of POMC precursor and its processed forms is secreted by a constitutive-like process which originates from immature granules. Transport of POMC and of a soluble bulk-flow marker, glycosaminoglycan chains, is dependent on cytosol, ATP and GTP hydrolysis, and is independent of Calcium ions. Serine/Threonine phosphatase inhibitors Calyculin A and okadaic acid block transport at a stage which precedes vesicle budding from the TGN. Inhibitors of Tyrosine kinases and phosphatases (Genistein, vanadate) also block an early event in granule formation and constitutive secretion.

S10-08

Glycosylation and disulfide bond formation of the β subunit are important for the maturation of Na,K-ATPase.

A. T. Beggah, P. Beguin, P. Jaunin and K. Geering, Institut de Pharmacologie et Toxicologie de l'Université, 1005-Lausanne

Co-translational modifications and the assembly of multimeric proteins are important for their functional maturation and expression. We have studied the role of two major co-translational modifications, namely coreglycosylation and S-S bond formation in the acquisition of an assembly competent configuration of the β subunit of Na,K-ATPase. Single, double and triple mutants, affected in consensus glycosylation sites or in S-S bond forming cysteine residues were prepared and expressed in Xenopus oocytes. Lack of glycosylation increased the half-life of unassembled β subunits and slightly affected the assembly efficiency with the α subunit and in consequence the cell surface expression of functional Na,K-pumps. On the other hand, β subunits lacking either one of the 3 disulfide bridges were degraded at a similar rate as unassembled wild type β subunits. Only the β mutant lacking the most N-terminal S-S bond in the ectodomain (CC1) can, though very inefficiently, associate with the α subunit. Compared to the 4-fold increase in new Na,K-pumps observed in oocytes expressing α and wild type β subunits, the CC1 mutant produced only about 1.4 times more functional pumps. Thus, co-translational modifications such as glycosylation and disulfide bridge formation influence the initial folding of the β subunit and in consequence the assembly with the α subunit necassary for the formation of functional Na,K-pumps expressed at the cell surface.

S10-09

Association with BiP is critical for the ER retention of single Na,K-ATPase subunits.

Paul M. Mathews, Ahmed T. Beggah, Bernard C. Rossier, and Käthi Geering; Institut de Pharmacologie et de Toxicologie, Université de Lausanne, CH-1005 Lausanne.

The α- and β-subunits of the Na,K-ATPase assemble to form an α/β heterodimer in the ER, which is then transported to the plasma membrane. When expressed alone, *Xenopus* subunits are retained in the ER of *Xenopus* oocytes. We have studied the role of the ER chaperone BiP (GRP78) in the retention and degradation of single Na,K-ATPase subunits both with anti-*Xenopus* BiP antibodies and by overexpressing the cloned *Xenopus* BiP cDNA. By co-immunoprecipitation, BiP can be shown to associate with either α or β. When a truncated BiP (BiPtr) lacking the ER retention signal KDEL was expressed, ER retention was relaxed: β, which is normally degraded in the ER, was less rapidly degraded, suggesting partial transport to a compartment other than the ER. While the endogenous oocyte α is not normally degraded in the ER, it was found to be degraded with BiPtr expression. We believe that this is due to the transport of endogenous α, associated with secreted BiPtr, out of the ER. Thus, association with BiP plays a role in the ER retention of unassembled α and β.

S10-10

IDENTIFICATION OF A NOVEL ENDOCYTOSIS SIGNAL CLOSELY RELATED TO THE KKXX ER-RETRIEVAL SIGNALS

Itin, C., Kappeler, F. and Hauri, H.-P. Biozentrum, University of Basel.

Membrane proteins often contain a sorting signal in their cytoplasmic tail that promotes their clustering into coated vesicles at a specific cellular site. ERGIC-53 contains a cytoplasmic ER-retrieval signal, KKFF, that mediates pre-Golgi localization. However, overexpressed ERGIC-53 is transported to the cell surface and rapidly endocytosed. Here we report that ERGIC-53 carries a previously undescribed endocytosis signal. Surprisingly, the signal was KKFF, and like the ER-retrieval signal required a C-terminal position. However, the minimal consensus sequence determined by substitutional mutagenesis (K-K/R-F/Y-F/Y) was significantly different from the ER-retrieval consensus (K-K-X-X). In fact, we provide evidence that internalization of VIP36, a protein that cycles between plasma membrane and Golgi, is mediated by a signal at its C-terminus that matches the internalization but not the ER-retrieval consensus sequence. Nevertheless, the relatedness of the two signals suggests that coatomer-mediated retrieval of proteins may be mechanistically more related to clathrin-dependent sorting than previously anticipated.

S10-11

Specific detection of transfected α1,3Fucosyltransferase (Fuc-T 5) in the Golgi apparatus by immunocytochemistry

L. Borsig and E.G. Berger, Institute of Physiology, University of Zurich, Switzerland
B. Bowen, CIBA-Geigy, Summit, USA

The fucosyltransferases (Fuc-Ts) constitute a family of transferases incorporating terminal fucose residues into glycoprotein or glycolipid glycans. Their putative cellular location is the Golgi apparatus. Immunocytochemical localization has been hampered by the lack of specific antibodies due to extensive homologies of Fuc-T III, V and VI at the protein level. Here we report the first immunocytochemical detection of a fucosyltransferase, in particular Fuc-T V. Plasmids encoding Fuc-T V were transfected into COS-cells and enzyme expression detected by using an antibody raised against a specific peptide stretch of Fuc-T V which is absent in the closely related Fuc-T III and Fuc-T VI. This antiserum was shown not to crossreact with the homologous Fuc-Ts transfected into COS-cells and tested for the presence of corresponding activities in COS cell lysates. Another specific antiserum to Fuc-T V was elicited by expressing the N-terminal part of the coding sequence as a fusion protein with ß-galactosidase in *E. coli*. Both antisera were characterized by ELISA, immunofluorescence and immunoprecipitation of Fuc-T V metabolically labeled in transiently transfected COS-cells.
These antisera will be used as tools to study developmental and spatial regulation of expression of Fuc-T V. Supported by the SNSF.

S10-12

Targeting of transfected ß1,4galactosyltransferase to the Golgi apparatus and the cell surface in COS-1 cells: role of the N-terminal 13 amino acids

A. Dinter and E.G. Berger, Institute of Physiology, University of Zurich, Switzerland

Earlier work has demonstrated that precursors of ß1,4galactosyltransferase (GT) exist as 42 kD (GT_s) and 44 kD (GT_l) forms corresponding to two initiation *met* sites separated by 13 amino acids. Data reported by other groups on differential targeting of these forms are conflicting. Here we show by immunofluorescence using monoclonal or protein-specific polyclonal antibodies that COS-1 cells transiently transfected with a plasmid encoding GT_l forms under the control of the CMV promoter expressed the recombinant enzyme both in the Golgi apparatus and on the cell surface. Conversely, expression of GT_s was restricted to the Golgi apparatus. Cell surface appearance of GT_l occurred in two aspects: as evenly distributed ectoenzyme on attached cells and in a condensed form on rounded (dead) cells suggesting toxic effects of overexpression on the cell surface. Transfection of the same plasmids into CHO cells which lack a replicating system did not lead to cell surface expression of GT suggesting that in order for the N-terminus to specify for cell surface expression a high level of expression is also required.
Supported by SNSF.

S10-13

Localization of the HIV-1 envelope glycoproteins during viral assembly

S. Wyss and M. Thali

Institute of Microbiology, University of Lausanne, Ch. des Boveresses 155, CH-1066 Epalinges, Switzerland

During budding retroviral capsids specifically acquire their envelope glycoproteins while excluding most cellular membrane proteins. Very little is known about the signals required for the specific incorporation of the envelope glycoproteins. It has been suggested that the retroviral capsid precursor competes with the cellular endocytic machinery in order to acquire these glycoproteins (E. Hunter, Seminars in Virology 5, 1994). The cytoplasmic tails of retroviral envelope glycoproteins contain motives which have been demonstrated to control the endocytosis of some cellular membrane proteins. We wanted to test whether such internalization signals found on the HIV-1 envelope glycoproteins are operational. In our preliminary localization studies, the HIV-1 envelope glycoproteins appear to be internalized. We will now try to confirm these results. The significance of our findings will be discussed.

S10-14

Biosynthesis of GDP-α-D̲-arabinopyranose in *Leishmania major* and *Crithidia fasciculata*.

Pascal SCHNEIDER[1,2] and Michael A. J. FERGUSON[2].
1: Institut de Biochimie, Université de Lausanne, CH-1066 Epalinges.
2: Dept. of Biochemistry, University of Dundee, Dundee DD1 4HN, UK.

D̲-Ara in its pyranose ring configuration is an uncommon sugar residue. It has only been described in a few trypanosomatids and is notably found as a terminal residue in the lipophosphoglycan of the parasite *L. major* and in a lipoarabinogalactan of *C. fasciculata*. In both cases the activated donor of the Ara_p residues is the sugar nucleotide GDP-α-D̲-Ara_p. Soluble extracts of biosynthetically labelled cells were analyzed using SAX HPLC, Dionex HPLC, chemical and enzymatic treatments. The arabinosyl residue of GDP-Ara was biosynthetically labelled using purified [2-^{3}H]D̲-Glc or [6-^{3}H]D̲-Glc, but not with [1-^{3}H]D̲-Glc, suggesting that the conversion of D̲-Glc to D̲-Ara involves the loss of the C-1 carbon atom. Pulse-chase labelling with synthethic [5-^{3}H]D̲-Ara indicated the following sequence of reaction: D̲-Ara->D̲-Ara-1-PO_4->GDP-D̲-Ara->acceptor glycolipid. A soluble arabino-1-kinase activity was partially purified from *C. fasciculata*. The arabino-1-kinase displayed a K_M^{Ara} of 24 μM, a K_M^{ATP} of 1.7 mM and was strictly dependent on MgATP as the donor substrate. However, competition studies indicated that arabino-1-kinase could accomodate D̲-Ara and D̲-Ara analogues (substituted on position 5 with methyl (L̲-Fuc) or hydroxymethyl (L̲-Gal) groups) as acceptor substrate. This arabino-1-kinase was utilized in the chemico-enzymatic synthesis of GDP-[5-^{3}H]D̲-Ara_p from [6-^{3}H]D̲-GlcN.

S10-15

THE ROLE OF THE ELECTROCHEMICAL MEMBRANE POTENTIAL IN PROTEIN INSERTION OF *E. COLI*

Andreas Kuhn, Dorothee Kiefer, Guoqing Cao, Ross Dalbey
Dept. of Applied Microbiology, University of Karlsruhe, D-76128 Karlsruhe, Germany and Dept. of Chemistry. Ohio State University, Columbus OH 43210.

We have studied two small membrane proteins that insert into the *Escherichia coli* membrane independent of the Sec translocase. The 44 amino acid long Pf3 coat protein translocates its amino terminal 18 residues aross the membrane. This region has two negatively charged residues that respond to the membrane potential which is positively charged at the outside. Substitution of these residues with positively charged ones inhibits translocation. Similarly, the central region of the 73 amino acid long M13 procoat that has a net charge of -3 translocates efficiently only across energized membranes. Substitution of these residues by neutral ones allows membrane translocation in the absence of the potential. Increasing the net charge of the region to -5 by inserting more negatively charged residues makes the membrane insertion even more dependent on the membrane potential than the wild type protein.

Calcium-binding proteins

S11-01

CALRETICULIN - A MULTIFUNCTIONAL PROTEIN

M. Michalak. Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada M5G 2S2

Calreticulin (CRT) is a Ca-binding protein found in a variety of cellular systems ranging from human to higher plants. CRT is a resident ER protein responsible for Ca storage/binding in the lumen of the membrane. The protein binds 1 mol of Ca with high affinity and >25 moles of Ca with low affinity. The two Ca binding sites are localized in different regions of the protein. CRT has also been found outside of the ER compartment, i.e. in the nuclear envelope, in the nucleus, in the cytotoxic granules in T-cells, in acrosomal vesicles, and on the cell surface. Most evidence indicates that CRT is a multifunctional protein. The protein binds to the DNA binding domain of the glucocorticoid and other steroid receptors *in vitro* and modulates steroid-sensitive expression *in vivo*. CRT interacts with the a-subunit of integrin effecting adhesion properties of different cells. The protein has also been shown to have an antithrombotic activity. We conclude that CRT is a multifunctional Ca binding protein which may play a fundamental role in the regulation of specific processes in different cellular compartments.

S11-02

Calretinin-22k has a specific nuclear binding site in stressed WiDr cells

Jean-Charles Gander, Eduardo Weruaga Prieto, Brigitte Hermann, Marco Celio and Beat Schwaller. Institut d'Histologie et d'Embryologie générale, Pérolles, 1705 Fribourg.

Calretinin (CR), a member of the EF-hand family of calcium-binding proteins, occurs in the colon adenocarcinoma cell line WiDr. In these cells an alternatively spliced transcript leading to a truncated form of calretinin, called calretinin-22k (CR-22k), has also been discovered. An antibody specific for the C-terminal amino acids of CR-22k localises this protein in the cytoplasm, as it is also known for the wild type protein.
Application of different types of stress (1,25-Dihydroxyvitamin-D3, heat shock) leads to a translocation of CR-22k into the nucleus of WiDr cells. We decided to investigate if CR-22k is uniformely distributed in the nucleus or if there is a specific interaction with nuclear components. Labelling of CR and CR-22k with fluorescein-isothiocyanate (FITC) allowed us to examine their precise localisation in the nucleus. Stressed and control cells were fixed and incubated with CR-FITC or CR-22k-FITC in a cytoplasmic-like buffer with or without Ca^{2+} (10µM or less than 40nM). In control cells, with or without Ca^{2+}, we never observed a specific interaction with nuclear components neither for CR-FITC or for CR-22k-FITC. In stressed cells, however, we detected a binding with CR-22k-FITC in the presence of 10µM Ca^{2+}. We conclude that there is a specific binding site for CR-22k in the nucleus of WiDr cells, which is available only in a Ca^{2+} dependent manner in stressed cells. Further experiments are aimed to identify this nuclear component.

S11-03

INCREASE OF MUSCLE RELAXATION SPEED BY DIRECT INJECTION OF PARVALBUMIN cDNA

Müntener, M., Käser, L., Weber, J. and Berchtold, M.W. Institute of Anatomy and Veterinary Biochemistry, University Zürich-Irchel, 8057 Zürich

Parvalbumin (PV) is a high affinity Ca^{2+}-binding protein found at high concentration in fast contracting/relaxing skeletal muscle fibres of vertebrates. It has been proposed that PV acts in the process of muscle relaxation by facilitating Ca^{2+} transport from the myofibrils to the sarcoplasmic reticulum. However, based on metal binding kinetics of PV *in vitro* this hypothesis has been challenged. To investigate the function of PV in skeletal muscle fibres, direct gene transfer was applied in normal and regenerating rat soleus muscles which do not synthesize detectable amounts of PV. Two weeks after *in vivo* transfection with PV cDNA considerable levels of PV mRNA and protein were detected in normal, and even higher amounts in regenerating, muscle. Twitch half relaxation time was significantly shortened in a dose dependent way in transfected muscles directly demonstrating the physiological function of PV as a relaxing factor in mammalian skeletal muscle.

S11-04

INACTIVATION OF CALPAIN IN INTACT CELLS

E.M. Vilei[1], J. Anagli[1], D. Guerini[1], M. Eppenberger-Eberhardt[2], H.M. Eppenberger[2], E. Carafoli[1]; Institutes of Biochemistry[1] and Cell Biology[2], Swiss Federal Institute of Technology (ETH), Zurich, Switzerland

The activation of calpain in different cell types was studied by raising the intracellular Ca^{2+} concentration $[Ca^{2+}]_i$ by incubating cells with the Ca^{2+} ionophore A23187 in the presence of increasing Ca^{2+} concentrations. The proteolysis of cytoskeletal proteins, PKC, and the plasma membrane Ca^{2+} pump (PMCA) (calpain substrates *in vitro*) was investigated with specific antibodies. Only the pump showed evident proteolysis in CHO transfectants expressing the PMCA4. Although no degradation of cytoskeletal proteins was detected, pre-incubation of cells with the cell-penetrating calpain inhibitor Cbz-Leu-Leu-Tyr-CHN_2 prevented the cytoskeletal rearrangement of dedifferentiated adult rat ventricular cardio-myocytes (ARC) in long-term culture, and cell division in cultures of HeLa, 293 and Cos-7 cells.

S11-05

Emerging functions in the annexin family

Upton, A.L., Edwards, H.C. and Moss, S.E.
Department of Physiology, University College London, Gower Street, London WC1E 6BT, U.K.

The annexins are a family of calcium-dependent phospholipid-binding proteins comprising at least thirteen unique gene products in mammals. Numerous functions have been ascribed to the annexins including roles in exocytosis, endocytosis and growth control.

We have recently investigated the proposed roles of annexin II and VI in exocytosis and endocytosis. Annexin II has been shown to efficiently stimulate the fusion of isolated chromaffin granules in µM Ca^{2+} and to restore secretory activity to permeabilised chromaffin cells that have been allowed to 'run down'. These observations suggest that annexin II functions to fuse secretory vesicles with the plasma membrane in response to elevated $[Ca^{2+}]_i$. We addressed this question by transfecting rat basophilic leukaemia cells with a reverse orientation cDNA encoding rat annexin II. The resulting antisense RNA caused abrogation of annexin II expression allowing examination of the secretory response in cells lacking this protein.

To study the role of annexin VI in endocytosis we created stable transfectants of human A431 cells expressing this protein. Although endocytosis was unaffected by the presence of annexin VI, we found these cells to be markedly restricted in their growth and less able to form tumours in nude mice. We conclude that although annexin VI has no role in endocytosis, the protein has a growth regulatory function, the mechanism of which is still unclear.

S11-06

STUDY OF CALRETININ-EXPRESSING CELLS IN THE PINEAL GLAND OF HUMAN AND MAMMALS USING IMMUNOHISTOCHEMISTRY AND IMMUNOBLOTING METHODS.

Novier A., Nicolas D., Krstic R., Institut d'Histologie & d'Embryologie, Bugnon 9, 1005 Lausanne

In a previous study (Novier & al, Experientia, 50:A81, 1994) we demonstrated the presence of calretinin-expressing cells in the pineal gland of human and mammals such as gerbils, rats, goats and cows, using immunohistochemistry. As calretinin antibody is considered as a specific marker for neurons (Andressen & al, Cell & Tissue Res, 271:181, 1993), we first thought these cells were a type of neuron. In fact, we found no morphological evidence of their neuronal nature. On the other hand, the calretinin-expressing cells seem to correspond anatomicaly to normal pinealocytes, containing calretinin.

To prove that it is calretinin which is shown in pinealocytes and not another pineal protein cross-reacting with our antibody against this calcium-binding protein, we used the Western Blot method. In all the pineal glands, we found a single 29 kDa band corresponding to calretinin.

Nevertheless, the nature of calretinin-expressing cells still needs further investigations.

S11-07

INTERACTIONS BETWEEN CRYSTALLINS AND FLUORESCENT SUBSTANCE LEAD TO CRYSTALLIN CROSSLINKING IN THE HUMAN LENS

H. Z. Malina & X. D. Martin

Laboratoire d' Ophtalmologie Expérimentale, Institut Universitaire de Pathologie, CH-1011 Lausanne

3-hydroxykynurenine (3-HK) is the product of tryptophan degradation in the human lens, and is part of the biochemical pathway leading to NAD formation. During cataract formation, 3-HK becomes a substrate for kynurenine aminotransferase (KAT) and is degraded to xanthurenic acid (XA). In this study, we look for biochemical consequences of XA formation. XA is oxidized giving 2 products, OXA and DOXA. The structures of these substances were established by IR and NMR. DOXA bound covalently to α- and β-crystallins. The conjugates of DOXA with α- and β- crystallins were isolated on Sephadex G-25. Crosslinking of α- and β-crystallins was observed on SDS-PAGE gels. Conjugates of DOXA and glutathione were formed in the presense of cataractous lens extract. All these events lead to cataract formation.

Swiss National Science Foundation 32-36058.92.

S11-08

Distribution and activity of calcineurin in rat tissues

Q. Su, M. Zhao*, E. Weber, H-P. Eugster and B. Ryffel
Institute of Toxicology of the University of Zürich and the Swiss Federal Institute of Technology, CH 8603 Schwerzenbach, *Institute of Pathology, Bern, Switzerland.
Keywords: Calcineurin, Cyclosporin, FK506

Calcineurin (CN), a Ca^{2+}/calmodulin-regulated phophatase 2B, plays an important role in many biological processes including T-cell signal transduction. In the present study, the distribution and activity of CN were investigated in rat tissues. CN has a wide tissue distribution as measured by enzyme-linked immunosorbent assay. CN concentrations are in the 0.3 μg/mg protein range in most tissues, while the brain contains 3 to 10 fold higher concentrations. Immunohistochemical analyses using a monoclonal antibody to CN B subunit reveals that CN is not evenly distributed but concentrated in specific cells in tissues especially in the brain, kidneys and testis. The specific enzymatic activity of CN in the tissues is in the range of 10 pmol/min/mg protein except for the brain, which has a six fold higher activity. The immunosuppressants CsA and FK506, but not rapamycin inhibit the enzymatic activity of CN derived from every tissue tested. Furthermore, transcripts and protein of the common CN-B and of the testis specific form of CN-B were analyzed in the tissues. The common CN-B transcripts and protein are found in all tissues. Transcripts for the 'testis specific' CN-B are also found in other tissues such as brain, lung, thymus and liver, while the protein is only detected in testis. This indicates that the testis specific CN-B gene expression is regulated at both transcriptional and posttranscriptional level. Together, the findings demonstrate that CN is a widely distributed protein phosphatase and its activity is regulated in a tissue specific manner.

S11-09

MYOSIN ISOFORMS AND CA-SENSITIVITY IN END-STAGE HEART FAILURE

Schaub, M.C. and Morano, I.*
Institute of Pharmacology, University of Zürich, CH-8057 Zürich, Switzerland; and *Max-Delbrück-Center, Cardiology, D-13122 Berlin-Buch, Germany

Isoforms of myosin heavy (MHC) and light (LC) chains were examined in explanted hearts of patients with end-stage heart failure (NYHA IV with ejection fractions of 12-34%), mostly dilated cardiomyopathies (DCM). Myosin with alpha-MHC has a higher ATPase activity than with beta-MHC. Right and left atria of six patients with DCM contained on average 48% and 44% beta-MHC as compared to 10% and 7% in five cases with compensated hemodynamics. Right and left atria of patients with DCM contained 10% and 8% of the ventricular VLC1 which does not occur normally in atrial tissue. Right and left ventricles always contain around 100% beta-MHC in disease and also in hemodynamically compensated cases. Right and left ventricles of patients with DCM contained 5.5% and 7.8% of the atrial ALC1 which does not occur normally in ventricular tissue. The Ca-sensitivity (ratio of pCa to isometric tension) of skinned papillary muscle bundles from left ventricles was higher in cases where the atrial ALC1 was present in different amounts in addition to the VLC1 (positive correlation with $r = 0.92$ at a significance level of $p < 0.01$). Force development is thus modulated by expression of different isoforms of type-1 LC in the human ventricle.

S11-10

INFLUENCE OF THE LIGHT CHAINS ON THE ENZYMATIC ACTIVITY OF FILAMENTOUS MYOSIN

Burgat, J.M., Schaub, M.C. and Cardinaud, R.*
Institute of Pharmacology, University of Zürich, CH-8057 Zürich, Switzerland; and *Institute of Biochemistry-Biophysics, University Paris XI, F-91405 Orsay, France

In striated muscle the myosin head domains in the crossbridges exert movement between the myosin and the actin filaments. The myosin light chains (LC1 and LC2) may be involved in myosin based regulation in addition to the Ca-switch for muscle contraction which operates via the regulatory proteins (troponin and tropomyosin) on the actin filament. Removing a short segment (7-19 amino acid residues) from the N-terminus of either type of LC has no effect on enzymatic activity in monomeric myosin nor in isolated myosin head domains. However, removal of the N-terminus from the LC enhances the Mg-ATPase activity of myosin in the filamentous state two-fold or more. The increase in catalytic activity by truncation of the LC can only be seen in filamentous myosin. It is furthermore salt dependent and disappears at KCl concentrations higher than 100 mM. It is known from X-ray diffraction that in muscle salt increase induces a transition of the crossbridges from an ordered state into disorder. So the ordered state of the head domains in the crossbridges of the myosin filament seems to be under the control of the LC. One can speculate that the N-terminal segment of both LC (LC1 and LC2) may be involved in modulating the equilibrium betwen ordered and disordered arrangement of the myosin head domains during rest and muscle activity.

S11-11

LOCALIZATION OF THE REGULATORY LIGHT CHAIN ON THE MUSCLE MYOSIN HEAVY CHAIN

Koch, D., Frank, G.* and Schaub, M.C.
Institute of Pharmacology, University of Zürich, CH-8057 Zürich; and *Institute of Molecular Biology and Biophysics, ETH-Zürich, CH-8093 Zürich, Switzerland

The regulatory light chain (RLC) of rabbit fast skeletal muscle myosin contains 169 amino acid residues and two unique cysteines in positions 128 and 157. A disulfide bridge can be induced between these two cysteines by DTNB in myosin in the absence of divalent metal ions. In the presence of either Ca2+ or Mg2+ the RLC can only be crosslinked to the myosin heavy chain (HC) by thiol-specific bifunctional reagents with a length of more than 6 Å (Fig. 1). Under identical conditions the RLC can also be crosslinked to the HC of myosin heads isolated by limited digestion with papain. Cys-128 of the RLC is much more reactive than Cys-157. Cys-815 was identified in the HC of isolated myosin heads as partner in the crosslinking reaction with the RLC. Cys-815 is directly located upstream of the IQ motif in the HC that is typical for calmodulin binding sites and is crosslinked to Cys-128 in the C-terminal portion of the RLC. Cys-815 lies in the HC binding region 808-826 for the C-terminal half of the RLC (Rayment et al., 1993). Downstream follows the stretch 825-842 which constitutes the HC binding region for the N-terminal half of the RLC. The entire binding region for the RLC on the myosin HC displays an unusually high degree of hydrophobicity. This may explain the aggregation in the neck region of myosin upon removal of the RLC.

S11-12

Disruption of the Gene Coding for Calmodulin in a Chicken B-Cell Line

Q.Ye and M.W.Berchtold, Institute of Veterinary Biochemistry, University of Zürich-Irchel, 8057 Zürich, Switzerland

Calmodulin (CaM) is a highly conserved Ca^{2+}-binding protein found in all eukaryotic organisms. Disruption or deletion of the single copy CaM gene in yeast and aspergillus suggested that CaM is essential for cell growth. In vertebrates CaM has been shown to be encoded by several genes making genetic experimentation difficult. The significance of CaM in the control of cell growth and physiology is therefore much less clear in vertebrates. To get insights into the role of CaM in higher eukaryotic cells *in vivo*, we disrupted the CaM gene by homologous recombination in a chicken B cell line (DT40) which carries out homologous recombination with high frequency. Disruption of both CaM alleles could be demonstrated by Southern blot and PCR analysis. CaM mRNA was no more produced in the knockout cells. However these cells exhibited normal growth characteristics and produced a protein with CaM immunoreactivity although with a several fold reduced abundance as compared to wild-type DT40. These findings indicate that there exists a back-up system to support cell growth in the absence of the targeted CaM gene. Studies are in progress to characterize the CaM immunoreactive material at a molecular level in knockout DT40 cells.

S11-13

THE MOUSE ONCOMODULIN GENE IS NOT REGULATED BY AN LTR PROMOTER

Frank Staubli and Martin W. Berchtold, Institut für Veterinär-biochemie, Universität Zürich-Irchel, CH-8057 Zürich.

The rat gene encoding oncomodulin (OM), a small calcium-binding protein, is under the control of a solo LTR derived from an endogenous intracisternal A-particle. The latter sequence is the only OM promoter analyzed so far. In order to study cell type specific OM expression in a species lacking LTR sequences in the OM locus we initially synthesized an OM cDNA from mouse placenta. By sequencing we found a 137 bp long 5' leader sequence which differed markedly from its rat counterpart but had high similarity to other mouse genomic sequences. Primers specific to this sequence in addition with primers specific for an exon 2/intron 2 sequence were used to screen a mouse ES cell genomic P1 library. One positive clone contained the whole OM gene, including intron 1 of 25 kb and a 5' flanking region of 27 kb lacking an LTR. Different lenghts of 5' flanking sequences will be analyzed for promoter/enhancer activity in transient transfections by a reporter gene assay.

S11-14

EXPRESSION OF S100 CALCIUM-BINDING PROTEINS IN NORMAL AND CANCEROGENIC HUMAN TISSUES

E. Ilg, B. Müller, B.W. Schäfer, G. Burg*, C.W. Heizmann*
Universität Zürich, Kinderspital, Abt. Klinische Chemie, Steinwiesstr. 75, 8032 Zürich
** Universitätsspital Zürich, Dermat. Klinik und Poliklinik, Gloriastr. 31, 8091 Zürich*

The calcium-binding proteins of the S100 family are candidates to mediate the physiological response to calcium signals in normal and transformed cells. S100A1, S100A2, S1000A4 and S100A6 e.g. are expressed in a tissue- and cell-specific manner. In order to study their biochemical properties, cDNAs were cloned into pGEMEX-plasmid and the recombinant proteins expressed in bacteria. In addition, we used these proteins to raise polyclonal antibodies in rabbit and goat. Antibodies were tested for crossreactivity with other S100 proteins and shown to be specific by western blotting. Subsequently they were used to study the expression pattern of individual S100 proteins in normal and cancerogenic tissues. Immunohistochemical stainings have been performed in nevi and various types of malignant melanomas as well as in melanoma cell culture preparations. Our results confirmed that S100A4 is up-regulated and S100A2 down-regulated in human cancerogenic tissues. We conclude that the use of specific antibodies to different members of the S100 family might be useful for a differential tumor diagnostic. To study the function of S100A4 and S100A2 we are now employing the yeast two-hybrid system to identify target molecules in vivo.

S11-15

PARVALBUMIN WILD-TYPE AND MUTANT CA^{2+}-BINDING PROTEINS WITH DIFFERENT METAL-BINDING PROPERTIES FOR STUDIES ON CA^{2+} HOMEOSTASIS AND CA^{2+} SIGNALING.

T.L. Pauls[1], I. Durussel[2], C. Andressen[1], V. Gotzos[1], B. Schwaller[1], M.W. Berchtold[3], M.R. Celio[1], J.A. Cox[2].
[1]Université de Fribourg, 1705 Fribourg, Switzerland; [2]Université de Genève, 1211 Genève, Switzerland; [3]University of Zürich-Irchel, 8057 Zürich, Switzerland.

Parvalbumin (PV) is an EF-hand helix-loop-helix Ca^{2+}-binding protein with two functional Ca^{2+}/Mg^{2+}-mixed metal-binding sites which bind Ca^{2+} with high affinity and Mg^{2+} with moderate affinity in a competitive manner. In order to investigate whether PV can influence Ca^{2+} homeostasis and modulate Ca^{2+}-dependent signaling within the cell we have produced, by recombinant technology, a variety of wild-type and mutant PVs with different metal-binding properties: The recombinant wild-type PV_{WT} and the mutant PV_{F102W} contain two Ca^{2+}/Mg^{2+}-mixed sites which are very similar to those of rat parvalbumin isolated from muscle. In contrast, the mutant PV_{-CD} has only a single Ca^{2+}/Mg^{2+}-mixed site with the same metal-binding properties as found in PV_{WT}, whereas the mutant PV_{-EF} only binds one Ca^{2+} or one Mg^{2+} with 10-fold reduced affinities and weak Ca^{2+}/Mg^{2+} antagonism, when compared to a Ca^{2+}/Mg^{2+}-mixed site. The mutant $PV_{-CD/-EF}$ binds neither Ca^{2+} nor Mg^{2+} and was constructed as a negative control protein. In a next step, we have started to overexpress these different PV wild-type and mutant proteins in cultured cells. First results with a non-neuronal adenocarcinoma cell line show a decrease in mitotic rate, changes in morphology from epitheloid to fusiform and an increase in motility of these cells after expression of PV_{WT}. No changes were found in cells expressing the Ca^{2+}-binding deficient mutant $PV_{-CD/-EF}$ when compared to untransfected control cells, indicating a Ca^{2+}-dependent action of PV_{WT}.

S11-16

THE ORGANIZATION OF THE HUMAN GENE OF THE SODIUM-CALCIUM EXCHANGER

A. Kraev and E. Carafoli, Laboratory of Biochemistry III, Swiss Federal Institute of Technology (ETH), CH-8092 Zürich

The intron-exon organization of the entire human Na/Ca-exchanger gene NCX1 and of the central part of the related gene NCX2 has been determined. The NCX1 gene is at least 75 kb long and consists of at least 12 exons, the two largest (the 2nd and the 12th) coding for the N-terminal half of the exchanger sequence and for the last two C-terminal transmembrane domains. They also code for the 3.3 kb 3'-untranslated region and account for more than 90% of the length of the mature mRNA. The remainder of the NCX1(NCX2) gene, coding for a putative cytoplasmic regulatory domain, is split into 9(7) small exons. In spite of the limited (65%) average homology of the two cDNAs, analogous exons are readily identified within this portion of the two genes based on their high (80-95%) pairwise homology and similar patterns of differential splicing in brain. Combinatorial potential of the exons, coding for the putative regulatory cytoplasmic domain, allows to generate more than 50 protein isoforms by differential splicing. The similar exon-intron organization of the "cardiac" (NCX1) and "brain" (NCX2) exchanger genes suggests their origin from the duplication and translocation of a common ancestral gene.

S11-17

Purification of μ-Calpain by a novel Affinity Chromatography Approach: New Insights into the Mechanisms of the Interaction of the Protease with Targets

Maurizio Molinari, Masatoshi Maki*, and Ernesto Carafoli#
Institute of Biochemistry, Swiss Federal Institute of Technology (ETH), 8092 Zurich, Switzerland

The aim of this study was to verify the presence domains of interaction between Calpain (CANP) and its preferred substrate in erythrocytes, the Ca^{2+}-ATPase of the plasma membrane. The presence of a Calmodulin (CaM)-binding motif is a common feature of a number of CANP substrates: it has been proposed that the evolutionary origin of the gene coding for CANP consists in the fusion of a gene coding for a thiol protease (Papain-like) and one coding for a Ca^{2+}-binding protein (CaM-like). The presence of a CaM-like domain in the CANP molecule and the presence of a CaM-binding domain in a number of substrates, led us to postulate that the two regions could be involved in the recognition process that preceeds the attack of the CaM-binding substrates by CANP. An attempt to purify μ-CANP from human erythrocytes by a new affinity chromatography approach in which the synthetic peptide C49 corresponding to the CaM-binding domain of the plasma membrane Ca^{2+}-ATPase was coupled to a CNBr-activated Sepharose matrix was successful. A supporting finding was obtained by infecting E.coli with a plasmid coding for the domain IV of human μ-CANP (the CaM-like domain (CaMLD) of the large subunit of the protease). The C49-Sepharose column retained the expressed CaMLD in a Ca^{2+}-dependent way, direct by showing the interaction between the CaM-binding domain of plasma membrane Ca^{2+}-ATPase and CaM-like domain of μ-CANP.

S11-18

Autoproteolysis Is Not an Obligatory Requirement for CANP Activation

Maurizio Molinari, John Anagli, and Ernesto Carafoli
Institute of Biochemistry, Swiss Federal Institute of Technology (ETH), 8092 Zurich, Switzerland

Interest in the function of calpain in physiology and in the development of pathological conditions is rapidly growing, but the precise role of the protease *in vivo*, and the processes leading to its activation in cells are still unclear. A widely accepted model claims that partial calpain-autolysis obligatorily precedes substrate cleavage.
In this work, the activation of calpain in the cell was investigated in human erythrocytes. The non-autolysed form of calpain was targeted to the erythrocyte membrane by increasing the Ca^{2+} concentration in the cells in a controlled manner. The membrane-bound calpain remained in the 80 kDa non-autolysed form in the presence of low cellular Ca^{2+} concentrations: under these conditions the preferred calpain substrates (the Ca^{2+}-ATPase and Band 3) were cleaved. That cleavage was due to calpain was confirmed by the finding that the two substrates were not degraded in the presence of the membrane permeable irreversible calpain inhibitor, Cbz-Leu-Leu-Tyr-CHN_2, nor when the (cellular) Ca^{2+} concentration was decreased to sub μM levels with EDTA. The findings have shown that calpain is active *in vivo* at lower Ca^{2+}-concentrations than previously assumed. They have also directly demonstrated that autolysis is not required for calpain activation within the (erythrocyte) cell environment.

S11-19

PARVALBUMIN IMMUNOREACTIVITY IN THE GERBIL HIPPOCAMPUS DURING THE CRITICAL PERIOD OF DEVELOPMENT OF SEIZURE SENSITIVITY.

A.L. Scotti, O. Bollag, G. Kalt, C. Nitsch, Anatomisches Institut der Universität, 4056 Basel.

The Mongolian gerbil (Meriones unguiculatus) is genetically predisposed to epilepsy. Seizure sensitivity evolves during the second month of life. Hippocampal disinhibition resulting from an increased number of GABAergic interneurons has been proposed as the basis for seizure sensitivity. Also, as a unique species difference the terminals of the gerbil perforant path contain the calcium binding protein parvalbumin (PV). PV is considered as a marker for the fast spiking subpopulation of GABAergic hippocampal interneurons and seems to affect activity potentiation at perforant path synapses in the gerbil. We analyzed the number and distribution of PV immunoreactive (PV IR) neurons in parasagittal sections of the perfusion-fixed gerbil hippocampus at postnatal day 30, 35, 40, 60 and determined the time of appearance of PV immunoreactivity in the terminal field of the perforant path. The number of PV neurons in CA1 and in AD increased progressively from day 30 to maximal values on day 40 to fall back to P30 levels on day 60. PV neurons counts in CA2 and CA3 fields remained constant with increasing age. The level of PV IR in the terminal zone of the perforant path was low on day 30 but evolved to the intense staining of the molecular layer typical for the adult animal within day 40, long before the time of onset of seizure sensitivity on day 50. On the contrary, a loss of PV IR neurons in CA1 and AD occurs as the age of onset for epileptic activity approaches.

S11-20

BIMODAL FREQUENCY DISTRIBUTION OF PARVALBUMIN-CONTAINING INTERNEURONS IN THE GERBIL HIPPOCAMPUS.

C. Nitsch, A.L. Scotti, F.-M. Nitsch, Anatomisches Institut der Universität, 4056 Basel.

In the hippocampal formation of the gerbil (Meriones unguiculatus) the calcium-binding protein parvalbumin (PV) is present in the perforant path from the entorhinal cortex to the stratum moleculare of the dentate area and the cornu ammonis. A possible relation of this species-specific feature to the seizure-sensitivity of the gerbils has been suggested. In addition, as in other mammals, PV is contained in a subpopulation of GABAergic interneurons. Characteristics and number of these neurons were analyzed in parasagittal vibratome sections of 27 aldehyde-fixed brains from untreated control gerbils. Total number and density of PV-immunoreactive neurons was estimated in CA1, CA3 and the dentate area in sections of defined laterality. Variations in neuron number and density were large in all fields. A graphic analysis of the frequency distributions revealed that, while in the dentate area and CA3 a Gaussian distribution was present, in CA1 the distribution was bimodal. Thus, concerning the PV-containing neurons in CA1, the present data set includes 2 different populations of gerbils. Since PV content has been considered to reflect neuronal activity, it seems possible that PV-expression in interneurons in CA1 reflects individual alterations in neuronal activity in the hippocampal formation as is the case in seizure-sensitive versus seizure-resistant gerbils.

S11-21

IMMUNOLOCALISATION AND CHARACTERISATION OF THE PRODUCT OF YKL056C, A YEAST GENE HOMOLOGOUS TO THE MAMMALIAN TRANSLATIONALLY CONTROLLED TUMOUR PROTEIN.

Schaller D.*, Federico S. *, Belet M.*, Sanchez J.-C.#, Hochstrasser D.# and Deshusses J.*. *Département de Biochimie, Université de Genève, CH-1211 Genève 4 and #Clinique Médicale, Département de Médecine, Hôpital cantonal Universitaire de Genève, CH-1205 Genève

The *Saccharomyces cerevisiae* gene YKL056C is coding for a polypeptide belonging to the family of the mammalian translationally controlled tumour protein. Members of this family have been found in mammals, birds and higher plants. At the moment, the function of these proteins is completely unknown. The gene has been deleted in a yeast clone. The mutant strain shows a slow growth. An antiserum has been obtained by injecting to rabbits the recombinant protein overproduced in *E. coli*. We have used the purified antibody to investigate the cellular distribution of the protein. By immunofluorescence, the protein was found to be localised in the cytoplasm. By western blot, we have found that this protein is an abundant component of the S100 fraction. Its similarity to a *Trypanosoma brucei* calcium-binding protein prompted us to investigate its metal binding properties.

S11-22

THE AMINO ACID SEQUENCE OF THE CD SITE DETERMINES ITS Ca^{2+}/Mg^{2+}-BINDING PROPERTIES IN CHIMERAS OF PARVALBUMIN AND ONCOMODULIN

I. DURUSSEL, T. L. PAULS, M. W. BERCHTOLD & J. A. COX
Department of Biochemistry, University of Geneva, and Institure of Veterinary Biochemistry, University of Zürich-Irchel

Here we test the working hypothesis that the affinity and the selectivity of a EF-hand site is determined by its sequence and by its tridimensional structure. As support we use the protein pair parvalbumin (PV) and oncomodulin (OM), which despite considerable structural homology, differ in the affinity and selectivity of metal-binding to their CD site: a high-affinity Ca^{2+}/Mg^{2+}-mixed site in PV and a low-affinity Ca^{2+}-specific site in OM. We created chimeras of PV and OM where the whole CD site (30 residues comprizing the α-helix-loop-α-helix) is exchanged for the corresponding one of the other protein, yielding two chimeras: PV_{OM}, for PV with a OM-like CD site, and OM_{PV}, for OM with a PV-like CD site. To ascertain the correct organization of the hydrophobic core, we replaced in these chimeras Phe_{102} by Trp. The latter mutation does not change the Ca^{2+}/Mg^{2+}-binding characteristics of the parent proteins, PV and OM.

Ca^{2+} and Mg^{2+} binding by flow dialysis and equilibrium gel filtration, as well as conformational changes in the Trp environment by fluorimetry and difference spectrophotometry, revealed that the primary structure of the CD site determines its Ca^{2+}/Mg^{2+} specificity in the chimeras, likely because in these constructs the influence of the rest of the protein (AB and EF segments) on the conformation of the CD site is believed to be the same as in the parent protein and therefore is the tridimensional structure of the CD site preserved in the host protein.

S11-23

THERMODYNAMIC AND MOLECULAR PROPERTIES OF THE INTERACTION BETWEEN AMPHIOXUS CALCIUM VECTOR PROTEIN AND ITS 26 KDA TARGET

T. V. PETROVA, M. COMTE, T. TAKAGI & J. A. COX
Department of Biochemistry, University of Geneva, Switzerland and the Biological Institute, Faculty of Science, Tohoku University, Sendai, Japan

Calcium vector protein (CaVP) of amphioxus, a 18 kDa calmodulin-like protein, is associated *in vivo* with a 26 kDa target (CaVPT), a multi-domain protein with one IQ and two IgII motifs. Isolated CaVP binds specifically two Ca^{2+} ions ($K'_{Ca1} = 4.9\ 10^6\ M^{-1}$ and $K'_{Ca2} = 7.3\ 10^3\ M^{-1}$). In the complex CaVP binds two Ca^{2+} with strong positive cooperativity ($n_H = 1.9$) and with distinctly higher affinity: $K'_{Ca1} = 2.4\ 10^5\ M^{-1}$ and $K'_{Ca2} = 1.0\ 10^8\ M^{-1}$. In the absence of Ca^{2+} the complex is stable, but Ca^{2+} binding to CaVP re-enforces the affinity between CaVP and CaVPT 70-fold. Each of the proteins interact with the hydrophobic probe TNS: CaVPT enhances its fluorescence 45-fold, $CaVP.Ca_2$ and metal-free CaVP only 10- and 5-fold, respectively. Complex formation between CaVPT and CaVP leads to a drastic reduction of the fluorescence enhancement, suggesting that a strong solvent-shielded hydrophobic core is formed. CaVP contains 2 highly reactional thiols ($k_{SH} > 0.3\ sec^{-1}$) for DTNB; CaVPT contains three thiols, two of them also with $k_{SH} > 0.3\ sec^{-1}$ in the native state and one buried thiol identified as Cys132. In the complex the reactivities of the exposed four thiols is dramatically reduced to $7.7\ 10^{-3}\ sec^{-1}$ (CaVP) and to $5.6\ 10^{-4}\ sec^{-1}$ (CaVPT). Ca^{2+} binding does not change the conformation of the complex, as probed with TNS and DTNB. In conclusion, isolated CaVPT and CaVP show the characteristics of highly dynamic, interactive proteins; complex formation leads to a rigid structure with a very stable hydrophobic core, likely yielding a non-interactive end product.

S11-24

Isolation of a YAC clone covering a cluster of nine S100 genes on human chromosome 1q21: rationale for a new nomenclature of the S100 calcium-binding protein family

R. Wicki[1], B. W. Schäfer[1], D. Engelkamp[2], M.-G. Mattei[3] and C. W. Heizmann[1]
[1]*Abteilung für Klinische Chemie, Kinderspital, Universität Zürich, Steinwiesstr. 75, 8032 Zürich;* [2]*Western General Hospital, Edinburgh, Scotland;* [3]*INSRM, Hôpital d'enfants, Marseille, France*

13 different S100 calcium-binding proteins have been isolated and characterized from human tissues by now. Several of these are differentially regulated in tumor cells with respect to normal cells, suggesting an involvement in tumorigenicity. Recently six S100 genes were found in a clustered form on chromosome 1q21. We now have isolated a YAC-clone from human chromosome 1q21, on which nine different genes coding for S100 proteins could be localized. Moreover, to address the question of gene regulation of S100 proteins located within the cluster, we isolated a lambda-clone containing the S100A2 gene (S100L), characterized its exon/intron structure and more than 2kb of the 5' upstream region. Finally, we mapped the gene coding for S100P to human chromosome 4p16 and completed the chromosomal assignment of all known human S100 genes. The clustered organization of S100 genes in the 1q21 region allows us to introduce a new logical nomenclature for these genes which is based on the physical arrangement on the chromosome (Genomics 1995, in press). The new nomenclature can be easily expanded to other species if a similar clustered organization of S100 genes should be found.

S11-25

IS CELL-CELL AGGREGATION IN MARINE SPONGES SPECIES-SPECIFIC?

J.Jarchow and M.M.Burger, Friedrich Miescher Institute, 4002 Basel

Cell-Cell aggregation in marine sponges is mediated by a class of Ca^{2+}-dependent extracellular matrix proteoglycans, termed aggregation factors (AF). In the case of the marine sponge *Microciona*, carbohydrate chains of the AF bind Ca^{2+}-independently to the cell, whereas the crosslinking of AFs occurs via homologous, Ca^{2+}-dependent carbohydrate-carbohydrate interactions. It is not known yet, if the crosslinking of the AF is species specific. We isolated the aggregation factors of two other sponge species and developed a cell free assay in order to study the interaction of the AFs only. AFs were coupled on either green or red fluorescent latex beads. Bead aggregates were allowed to form in the presence of 10mM Ca^{2+} (physiological to sea water) on a rotary shaker and the distribution of the different beads within the aggregates was studied by confocal microscopy. Our results indicate that the aggregation of whole AFs is species specific in one case, in the other, a species preference can be observed.

S11-26

ANTISENSE OLIGONUCLEOTIDES DIRECTED AGAINST THE CARDIAC NA-CA EXCHANGER mRNA

B. Schwaller[1], P. Lipp[2] and E. Niggli[2]. [1]Dept. of Histology, University of Fribourg, [2]Dept. of Physiology, University of Bern, Switzerland.

The Na-Ca exchange is responsible for Ca removal in cardiac myocytes and may contribute to excitation-contraction coupling. The investigation of this transporter has been difficult because no specific inhibitor is available. We have constructed a phosphorothioate antisense oligonucleotide (19-mer) directed against the 3' nontranslated region of the rat cardiac Na-Ca exchanger mRNA. Cell cultures were exposed to 3 μM oligonucleotide and single myocytes dialyzed with a mixture of two Ca indicators and the "caged Ca" compound DM-nitrophen. We performed experiments on control and exposed cells after inhibiting the Ca stores. To estimate the activity of the Na-Ca exchange, the exchange current (I_{NaCa}) was measured with the voltage-clamp technique while Ca_i was simultaneously recorded with a confocal microscope. After 24 hours, most exposed cells showed neither a detectable I_{NaCa} nor an increase of Ca_i upon Na_o removal. Flash photolytic Ca_i concentration jumps persisted for minutes without significant decay, whereas in control cells resting Ca_i was reached after ≈ 6 sec. These results suggest that the transport function was almost completely suppressed and that antisense oligonucleotides represent a useful tool to investigate the cellular and molecular properties of the Na-Ca exchanger. Supported by SNF.

S11-27

ENSHEATHING PATTERN OF CALCIUM-BINDING PROTEINS-CONTAINING AXONS IN THE RAT CENTRAL NERVOUS SYSTEM

E. WERUAGA PRIETO, P. EGGLI*, M.R. CELIO
Institut d'Histologie et d'Embryologie générale, Université CH-1705 Fribourg.
*Anatomisches Institut, Universität CH-3012 Bern.

The sheet-like processes of oligodendrocytes wrap themselves spirally around central axons, thus forming the myelin sheath. A single oligodendrocyte may donate internodal segments of myelin to each of 20-30 or more adjacent axons. It is not known, if one oligodendrocyte picks out axons randomly or if it prefers axons with a certain diameter or with a certain chemical specificity. We have studied this last possibility by combining intracellular injection of a fluorochrome, and immunohistological detection of calcium-binding proteins (CaBP's), markers for the axons of certain subpopulations of nerve cells.

Lucifer Yellow was injected into oligodendrocytes of the optic nerve, corpus callosum or cerebellum of living and fixed rat brain slices. The oligodendrocytes were identified by their morphology. Slices containing the injected cells were immunostained for parvalbumin (PV) or calretinin (CR) using second antibodies labelled with Texas red. Using confocal laser-scanning microscopy combined with a three-dimensional reconstruction programm, the relationship between oligodendrocyte processes and axons positive for one of the CaBP's was determined. For ultrastructural confirmation, single, Lucifer-Yellow injected oligodendrocytes were photoconverted, and the tissue was processed for electron microscopy.

The injected oligodendrocytes show the classic morphology, that is of a small cell body and few processes running parallel to the course of the axons. The relation between glial processes and positive axons can be easily appreciated in confocal laser-scanning images and a primary ensheathing pattern was established. Single oligodendrocytes of the optic nerve wrap preferentially CR-positive axons but not PV axons.

Nevertheless, the confirmation of the hypothesis that oligodendrocytes choose their axonal targets according to chemical cues awaits further work including the injection of a larger number of these cells.

S11-28

THE NATURE OF THE INTERACTION BETWEEN CALPAIN AND ITS ENDOGENOUS INHIBITOR CALPASTATIN

John Anagli and Ernesto Carafoli, Institute of Biochemistry, Swiss Federal Institute of Technology (ETH), CH-8092 Zurich, Switzerland

Calpain is a non-lysosomal cysteine proteinase to which various functions have been attributed based on *in vitro* proteolysis experiments. The exact role(s) of the enzyme *in vivo* is, however, still not clear. The activity of calpain is under a very tight regulation which involves modulation by Ca^{2+}, translocation from the cytosol to the cell membrane, inhibition by calpastatin and perhaps other mechanisms yet to be discovered. The specific inhibitor calpastatin shares no sequence or structural similarity with any of the known protein proteinase inhibitors. A 27 residue synthetic peptide derived from exon 1B of the calpastatin gene inhibits the enzyme with a K_i of about 40 nM. In order to understand the nature of the calpain-calpastatin interaction, mutated and photoactivatable versions of the inhibitory peptide have been used to determine the type(s) and sites of protein-protein/peptide interaction.

Drosophila genes and neurogenesis

S12-01

ANALYSIS OF *REPO*, A GENE INVOLVED IN GLIOGENESIS

Joachim Urban‡, Daniel Halter†, Christof Rickert‡, Sarbjit S. Ner†, Kei Ito‡, Andrew A. Travers† and Gerhard M. Technau‡

‡ Insitut für Genetik, Abt. Zellbiologie, Unversität Mainz, Saarstrasse 21, 55099 Mainz, Germany
† MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, England

To elucidate the process of glial development we were looking for gliaspecific genes in the embryo of *Drosophila melanogaster*. Here we describe the expression and phenotypic characterization of such a gene encoding a homeodomain protein that is nearly exclusively expressed in most glia or closely related cells in the CNS and PNS except the midline glia.
Using the anti-repo antibody we were able to trace some of the glial cells back to their presumable origin. Embryos which are homozygous for null alleles of the protein exhibit late developmental defects within the embryonic nervous system including a reduction in the number of glial cells, defasciculation of axons at late embryonic stages and the inhibition of nerve cord condensation. The expression of an early glial-specific marker is unaffected in such homozygotes. By contrast the expression of late glial-specific markers is either substantially reduced or absent.
We conclude that although the protein is not required for the initial determination of glial cells it is essential for their further differentiation and for the maintenance of glia function.

S12-02

IRREGULAR CHIASMS C - ROUGHEST: A RECOGNITION MOLECULE IN THE VISUAL SYSTEM OF *DROSOPHILA*

Karl-Friedrich Fischbach
Institut für Biologie III, Schänzlestr. 1, D-79104 Freiburg

Loss of function mutations in the *irreC-rst* gene lead to axonal projection defects in the outer and inner optic chiasms of *Drosophila melanogaster* (Boschert et al., J. Neurogenetics 6, 153-171; 1990). The gene codes for a member of the immunoglobulin-like superfamily of cell adhesion molecules (Ramos et al., Genes & Development, 7, 2533-2547; 1993). The nearest relative of the IRRE C-RST protein known so far is the chicken BEN/DM-GRASP/SC1 protein (e.g. Pourquie et al., PNAS. USA 89, 5261-5265; 1992). The *irreC-rst* gene is temporally and spatially highly regulated. In the developing visual system the 104 kD glycoprotein is expressed on a small subset of newly outgrowing axons that project through the optic chiasms. Later in development it is confined to distinct synaptic layers of the visual neuropiles and is downregulated before eclosion. In order to test our hypothesis that IRRE C-RST protein expression pattern directs axonal growth and target recognition, we misexpressed a major cDNA of the locus. When IRRE C-RST is globally expressed in the optic lobe, a phenocopy of the optic lobe null phenotype results, i.e. loss and gain of function phenotypes are similar. This result is in accordance with our hypothesis that the restricted distribution of IRRE C-RST is essential for oriented axonal growth. By cell type specific gene targeting more specific wiring defects can be achieved.
With regard to the protein's cellular function we could show that it is involved in reorganizing membrane contacts in the eye imaginal disc. Although able to mediate homophilic binding in vitro, its in vivo function is more complex and evidence for heterophilic as well as repulsive functions does exist. Our work is supported by the DFG (Fi 336/7-1).

S12-03

SECRETED FACTORS AFFECTING CELL DETERMINATION AND AXON GUIDANCE IN THE VISUAL SYSTEM OF *DROSOPHILA*

Brunner, A., Pollack, I, Baumgartner, S.[1] and Schneuwly, S.[2]
Lehrstuhl Genetik, Biozentrum, Universität Würzburg, D-97074 Würzburg.
[1]Friedrich Miescher Institut, 4002 Basel.
[2]Institut für Zoologie, Universität Regensburg, D-93040 Regensburg.

Secreted molecules play an important role in cellular communication and axon guidance. We are using the visual system of *Drosophila* as a model system to study the effects of secreted molecules on the development of neuronal cells. One of the genes analyzed in our lab (*gil*) encodes a secreted protein which, as a lateral inhibitor, specifically affects cell fate decisions and axon guidance in the visual system.
Another class of molecules involved in the signalling pathway in axonal outgrowth belongs to the extracellular matrix. Using genetic tools, we are investigating the role of these molecules during visual system development.

S12-04

EMBRYONIC BRAIN DEVELOPMENT IN *DROSOPHILA* .

H. Reichert, S. Therianos, S. Leuzinger, F. Hirth, and C. S. Goodman, Institute of Zoology, University of Basel and Department of Molecular and Cell Biology, University of California, Berkeley

We are interested in the molecular mechanisms involved in building a complex brain. To this end we are studying embryonic brain development in *Drosophila* where powerful genetic and molecular genetic technology is available. During embryogenesis in *Drosophila*, complex cerebral hemispheres are formed and become linked to the ventral nerve cord by paired brain connectives, which interconnect the initially separated procephalic and ventral neurogenic regions. The cerebral hemispheres are interconnected by a primary embryonic brain commissure, which is pioneered by a pair of axons that navigate along a midline intercerebral glial bridge. The differentiation of this brain commissure depends on the *comm* gene and other related genes. Thus, in *comm* mutants the brain commissure is severely reduced, and only a small subset of commissural fascicles are formed. The formation of the paired brain connectives depends on the *sim* gene and on other related *spitz*-class genes. Thus, in *sim* mutants paired connectives do not form, and aberrant ectopic midline interconnections between brain and ventral nerve cord are established by axons which navigate along the midline stomatogastric nervous system. Our future goal is to carry out a comprehensive molecular genetic analysis of the set of genes involved in embryonic and postembryonic brain development in *Drosophila*. (Supported by SNSF and HHMI).

S12-05

CONTROL OF PHOTORECEPTOR CELL FATE BY COMPONENTS OF THE SEVENLESS SIGNALLING CASCADE IN *DROSOPHILA*

E. Hafen, D. Brunner, B. Dickson, M. Dominguez, K. Dücker, N. Oellers, A. van der Straten. Zoologisches Institut, Universität Zürich, Winterthurerstr. 190, 8057 Zürich.

The decision between cone cell fate and R7 cell fate in the developing eye of *Drosophila* depends on the activity of the Sevenless (Sev) receptor tyrosine kinase (RTK). The signal from the Sev RTK is transduced by a set of highly conserved signalling components including Drk, Sos, Ras1 and Raf. To identify loci required for signal transduction downstream of the Raf kinase we have performed several genetic screens for dominant suppressors of the rough eye phenotype caused by the constitutive activity of Raf in the R7 equivalence group. Results will be presented showing that the Sev signal is mediated by the phosphorylation of two ETS transcription factor, PointedP2 and Yan, by MAP kinase. The characterization of a dominant suppressor of active Raf led to the identification of *phyllopod* (*phyl*), which encodes a novel nuclear protein required for the development of a subset of photoreceptors and other sensory organs. *phyl* expression in the R7 equvalence group is regulated by the Sev pathway. Since *phyl* expression is sufficient to specify R7 cell fate in the absence of an upstream signal it represents a novel cell fate selector gene.

S12-06

Function of the Drosophila Pax-6 homologue, *eyeless*, in eye morphogenesis. Patrick Callaerts, Georg Halder and Walter J. Gehring, Dept. of Cell Biology, Biozentrum, University of Basel, CH-4056 Basel

The gene *eyeless (ey)* encodes a paired domain- and homeodomain-containing protein which is highly homologous to the mouse gene *Small eyes* and the human gene *Aniridia* (Quiring et al, Science 265, 785). The phenotype observed in two *ey* mutants is caused by the insertion of a doc (ey^2) and a blastopia (ey^R) transposable element in an eye-specific enhancer element resulting in the loss of *ey* expression (Walldorf and Gehring, in preparation). The gene is first expressed transiently in the ventral nerve cord and the brain of the embryo and later in larvae, pupae and adults. It is also expressed in the eye anlagen of the embryo and in the eye-discs of the larva. In the third instar larval eye-disc, it is expressed anterior to the morphogenetic furrow, i.e. very early in eye morphogenesis. The tight parallelism between gene expression and development of eye primordia suggested an early and probably very important function of *eyeless* in these events. The analysis of *eyeless* expression in different mutants affecting eye development has corroborated that *eyeless* is indeed very high up in the genetic hierarchy leading to eye formation. Direct evidence for the importance of *ey* in eye morphogenesis comes from the **induction of ectopic eye tissue** through a heatshock-induced overexpression or a GAL4-UAS mediated misexpression of the gene.

Eye structures with facets, lenses, interommatidial bristles and pigment cells have been induced in all three leg discs, as well as in wing and antennal discs. We are currently analyzing whether these eye structures are functional.

S12-07

ANTENNAL LOBE METAMORPHOSIS IN *DROSOPHILA*

M. Tissot, N. Gendre, A. Hawken, K.F. Störtkuhl & R.F. Stocker
Zoologisches Institut, Universität Freiburg, CH-1700 Freiburg

The adult antennal lobe (AL) emerges from a larval precursor, which axonal tracing suggests to be the target of larval olfactory sensilla. Bromodeoxyuridine (BrdU) incorporation at different developmental stages shows that most of the AL interneurons originate from neuroblasts that begin to divide in the 2nd instar. In the lobe region, a maximum of 10-12 pairs of neuroblasts undergo mitosis in the late 3rd instar, while glial divisions peak only 12 hr after pupariation (AP). The soma position of interneurons in the AL cortex is not correlated to their birthdates.
The first neurons are recognized in the antennal disc by the afferent-specific mab *22C10* 3 hr AP. Their axons fasciculate with persisting fibers in the larval antennal nerve. Two afferent-specific GAL4 enhancer trap lines (*14* & *116*) isolated in our lab become expressed 24 hr AP in different subsets of antennal neurons. Label extending along the antennal nerve shows that at this time afferents have already reached the ipsilateral AL and 36 hr AP the contralateral AL. 48 hr AP, the labeled antennal elements can be assigned to large basiconic sensilla in line *14* and to trichoid and small basiconic sensilla in line *116*. In the AL, different subsets of glomeruli are stained by the two lines, confirming anatomical data. GAL4 expression persists to the adult in line *14*, but disappears in line *116*. Two other GAL4 lines enabled us to study the differentiation of AL interneurons. Line *7B* is expressed in unilateral local interneurons (LI), while line *78* labels a new, bilateral type of LI with arborizations in only few glomeruli. The key features of this pattern are present already 48 hr AP. BrdU labeling allows us to determine the birthdates of these interneurons.

S12-08

THE *DROSOPHILA DACHSOUS* (*DS*) GENE, A NEW MEMBER OF THE CADHERIN SUPERFAMILY

Doris Brentrup[1], Hillary F. Clark[2], Kay Schneitz[1], Allan Bieber[3], Corey Goodman[2], and Markus Noll[1].
[1]Institut für Molekularbiologie II, Universität Zürich, CH-8057 Zurich, Switzerland;
[2]Howard Hughes Medical Institute, Dept. of Molecular and Cell Biology, University of California, Berkeley, CA 94720, U.S.A.;
[3]Dept. of Biological Sciences, Purdue University, West Lafayette, IN 47907, U.S.A.

A DNA segment encoding cadherin domains was amplified by PCR from *Drosophila* genomic DNA. The PCR product, located in 21D, originates from the *ds* gene, which was cloned by extending a chromosomal walk from 21C1,2 proximally into the 21D1,2 region. Mapping *ds* cDNAs to the overlapping clones of chromosomal DNA shows that the primary *ds* transcript is more than 65 kb in length. The 12.4-kb cDNA consists of a 10.7-kb ORF in 12 exons. The spatial and temporal distribution of *ds* transcripts were analyzed by DIG-*in situs* on whole mount embryos and larval discs. The first *ds* transcripts are detectable during gastrulation in a pair-rule striped pattern. At later stages, it is expressed mainly in invagination sites (*eg*, in tracheal pits and leg disc primordia) and segmental folds. In larvae, expression is found in all discs and in the brain. The *ds* gene encodes a transmembrane protein with 27 tandemly repeated cadherin domains. The cytoplasmic portion of the Ds protein has sequence similarity to the cytoplasmic domains of classic vertebrate cadherins, which were shown to interact with β-catenin linking the cadherins to the cytoskeleton.

S12-09

ROLE OF *POX MESO* IN *DROSOPHILA* MUSCLE DEVELOPMENT

Hong Duan, Weimin Fu, Erich Frei, and Markus Noll.
Institut für Molekularbiologie II, Universität Zürich, CH-8057 Zürich, Switzerland.

The *Drosophila* gene *pox meso* (*poxm*) encodes a transcription factor containing a paired-domain and is expressed with a segmental periodicity in the embryonic somatic mesoderm (Bopp *et al.* 1989. *EMBO J.* 8, 3447-3457). Since the embryonic somatic mesoderm later develops into body musculature, *poxm* might be involved in the development of larval somatic muscles. As no *poxm* mutant alleles are available, the function of *poxm* is unknown. However, several deficiencies exist that uncover the *poxm* gene. Yet even the smallest deficiency, obtained by the heteroallelic combination of *Df(3R)dsx5* and *Df(3R)p13*, deletes several genes, including *poxm*. Preliminary results, obtained by examining the morphology of transheterozygous *Df(3R)dsx5/Df(3R)p13* embryos, indicate that the esophagus and proctodeum fail to develop, the arrangement of the body wall muscles is changed, and the embryos die before hatching. Since several loci are deleted in these embryos, it is unknown whether these changes result from the lack of *poxm* function. Therefore, our work focuses on the search for *poxm* mutants. Several P-element insertions in the region uncovering the *poxm* locus have been isolated by screening a collection of P-element insertions on the third chromosome. One of them is located 5.5 kb downstream of the *poxm* transcription unit and hence was mobilized to generate imprecise excisions to obtain a deletion that removes at least part of the *poxm* gene.

S12-10

USE OF A POLYCLONAL ANTI-CHOLINE ACETYLTRANSFERASE ANTIBODY TO ANALYZE THE DROSOPHILA ENZYME EXPRESSED IN HUMAN NEUROBLASTOMA CELLS.

Pahud, G., Salem, N., Medilanski, J., Pellegrinelli, N. and Eder-Colli, L.**
Department of Pharmacology, CMU, 1211 Geneva 4

Choline acetyltransferase (ChAT) activity exists in at least two forms inside cholinergic neurons; that is, hydrophilic and amphiphilic activities. Aiming to investigate the way in which amphiphilic enzyme is formed, we expressed a cDNA clone coding for *Drosophila* ChAT in a non-cholinergic human neuroblastoma cell line (SK-NBE). Also, we developped a rabbit polyclonal antibody directed towards a recombinant fragment of ChAT (48 kDa polypeptide) that was prepared using a 1.3 kb fragment located in the C-terminal part of the coding region of *Drosophila* ChAT cDNA. Hydrophilic and amphiphilic ChAT activities were expressed in transfected cells. The antibody bound to protein A sepharose was able to immunoprecipitate both hydrophilic and amphiphilic ChAT activities extracted from *Drosophila* heads as well as from transfected neuroblastoma. On western blots of SDS-PAGE containing total, hydrophilic or amphiphilic enzyme activity, the antibody detected a polypeptide of 75 kDa, whenever native or recombinant enzyme was analyzed. Thus, the two forms of *Drosophila* ChAT appeared to have same molecular weight. Immunohistochemical analysis of transfected cells that had been acetone fixed, resulted in a specific staining which appeared as a fine network around nucleus. We are presently investigating whether or not amphiphilic ChAT is interacting with a membrane protein which might anchor it to membranes.

S12-11

THE *dunce* GENE PRODUCT MIGHT BE PHYSIOLOGICALLY INVOLVED IN THE SEX-PEPTIDE RESPONSE IN *Drosophila melanogaster*

Irène Fleischmann, Brigitte Dauwalder*, Tracey Chapman**, Bea Cotton and Eric Kubli

Zoologisches Institut, Universität Zürich-Irchel, Zürich, Switzerland
* Baylor College of Medicine, Dept. Cell Biology, Houston, TX 77030, USA
**The Galton Laboratory, University College London, London NW1 2HE, UK.

Sex-peptide (SP), a 36 amino acid long peptide, is synthesised in the male accessory glands of *D. melanogaster.* During copulation it is transferred into the female. Injected into virgin females, or ectopically expressed, SP induces reduced receptivity and increased oviposition, the two post-mating reactions observed after copulation. The behavioral changes imply that the nervous system is involved in the females´ reaction to SP. The response of the learning mutant *dunce* to SP injections was investigated. The affected gene is expressed predominantly in the mushroom bodies, where it affects the cAMP levels. SP injection into *dunce* females fails to elicit the responses described above. We used transgenic *dunce* females carrying a hsp-*dunce* construct to test whether the *dunce* gene product is physiologically required for induction of the SP response at the time of injection. Thus far we have tested a transgenic line carrying a rat *dunce* homologue as a rescue construct. In comparison with controls we observed a partial rescue in heat-induced *dunce* transgenic females injected with SP. Since *dunce* gene expression is strongest in the mushroom bodies, we will discuss the possible involvement of the mushroom bodies as a part of the SP-response-system in the female.

S12-12

MOLECULAR BASIS OF INTRA-CISTRONIC COMPLEMENTATION IN THE *PASSOVER* LOCUS

Erich Frei[1], Sibylle Burger[1], Santosh N. Krishnan[2], Gary P. Swain[2], Abraham P. Schalet[2], and Robert J. Wyman[2]
[1]Institut für Molekularbiologie II, Universität Zürich Irchel, 8057 Zürich
[2]Department of Biology, Yale University, New Haven, CT 06511

The *Passover* mutation disrupts specific synaptic connections in the Giant Fiber (GF) System of *Drosophila*. In the CNS of the adult, and of the pupa during GF synapse formation, *Pas* transcripts are consistently expressed in the GF and a large thoracic cell in the location of its postsynaptic targets. *Pas* is homologous to other neural genes: *ogre* (*optical ganglion reduced*), required for post-embryonic neuroblast development in *Drosophila*, and *unc-7*, which, when mutated, alters the connectivity of a few neurons in *C. elegans*. A group of allelic lethal mutations exhibit a complex pattern of complementation with *Pas* and its alleles. Whereas all heterozygotes between these lethals and *Pas* are viable, some complement while others fail to complement the neural defect of *Pas*. We show that the lethal and neural functions of this locus are encoded by two proteins that have similar but distinct N-terminal domains joined to a common C-terminal portion. Neural-specific and lethal-specific mutations map to the unique portions, while neural lethal mutations map to the common domain. Thus, transheterozygous combinations of lethal over neural alleles can result in production of both proteins and thereby demonstrate intra-cistronic complementation. This mechanism for intra-cistronic complementation does not require the interaction of different subunits to create a wild-type protein.

S12-13

INSECT EVOLUTION: CONSERVATION OF THE *SEX-LETHAL* GENE.

A. Dübendorfer[1], D. Hilfiker-Kleiner[2], M. Scott[2], D. Bopp[1], M. Meise[1], J.C. Lucchesi[2] and R. Nöthiger[1].

[1] University of Zürich, Zoology Institute, Winterthurerstr. 190, 8057 Zürich
[2] Emory University, Dept. of Biology, 1510 Clifton Road, Atlanta, U.S.A.

We investigate the evolution of sex-determining mechanisms in insects by comparing the genetic control of this process in two dipteran species, *Drosophila melanogaster* and *Musca domestica*. These species use different sex-determining mechanisms which, however, show some strategic parallels: In *Drosophila*, the ratio of X-chromosomes to autosomes constitutes the primary sex-determining signal that controls a key gene, Sxl. In *Musca*, the male-determiner *M* on the Y chromosome is thought to be the primary signal that controls the key gene *F*.

To see if the *Sxl* gene has been conserved in *Musca* and *Drosophila*, we searched genomic and cDNA libraries of *Musca* with PCR amplified sequences designed after the *Drosphila Sxl* gene. We isolated sequences from *Musca* cDNA with up to 90% similarity to the *Drosophila Sxl* gene. RNA analyses, however, did not reveal any sex-specific transcripts in *Musca* so far. Antibodies against *Drosophila* SXL protein recognize a protein in *Musca* that corresponds in size to the smaller of the two isoforms expressed in *Drosophila* females. In contrast to *Drosophila*, however, the same antigen is also detected in male tissues of *Musca*. We conclude that the *Sxl* gene has been conserved, but in *Musca* is not sex-specifically regulated and may therefore not have the same sex-determining functions as *Sxl* has in *Drosophila*.

S12-14

THE GERMLINE AFFAIRS OF A SOMATIC SWITCH GENE CONTROLLING SEXUAL DEVELOPMENT.

Daniel Bopp, Benjamin I. Arthur, Huang He and Rolf Nöthiger
Zool. Institut der Uni Zürich, Winterthurerstr. 190 CH-8057

In Drosophila, the number of X chromosomes constitutes the signal that directs the sexual fate of the developing embryo. The sole target of this signal is the gene *Sex-lethal* (*Sxl*) which becomes activated in XX female cells, but remains inactive in XY or XO male cells. Female differentiation requires the continuous activity of *Sxl* while this gene must always be *off* to allow male development. In the soma, XX cells become male when depleted of *Sxl* activity, and XY cells become female when *Sxl* is ectopically activated

Less is known about the role of *Sxl* in the germ line where it is needed for differentiation of female germ cells. We have investigated this question by ectopically expressing SXL protein in early male germ cells. In contrast to the observations in the soma, *Sxl* does not alter the sexual fate of primary spermatocytes. Moreover, despite the large amounts of SXL protein produced, these cells proceed with spermatogenesis to become functional sperm. Female germ cells that are defective for *Sxl* can be rescued by the same transgene indicating that the protein expressed by this construct is fully functional in the female germ line. From these and other data we conclude that *Sxl* does not operate as a master switch gene in the germ line. We propose a model in which *Sxl* controls one of several subordinate sex differentiation pathways in oogenesis. Recent data on the search and characterization of other components in this pathway will be presented.

S12-15

MOLECULAR CHARACTERIZATION OF *VIRILIZER*, A POSITIVE REGULATOR OF *SEX-LETHAL*

Markus Niessen, Roger Schneiter and Rolf Nöthiger
Zoologisches Institut der Universität Zürich, Winterthurerstr.190, CH-8057 Zürich

In *Drosophila*, the gene *Sex-lethal* (*Sxl*) acts as the master switch in sexual development. When it is on, cells will assume a female fate; when it is off, male development will ensue. The initial activity state of *Sxl* is selected by a chromosomal signal (the X:A ratio) and becomes then fixed by a self-regulatory feedback loop. To sustain its activity in females, *Sxl* requires its own products in concert with other components, one of which is *virilizer* (*vir*). Our group has genetically characterized this gene and demonstrated that *vir* is necessary for female-specific expression of *Sxl* as well as for vital processes unrelated to sex.

A molecular analysis has been initiated to determine how *vir* executes its functions in development. A genomic region of 180 kb harboring *virilizer* has been isolated, and a 10 kb fragment from this region has been shown to contain the necessary sequences for all of the virilizer functions. We have also isolated putative *vir* cDNAs which we are currently analyzing. We will use our genetic and molecular data to propose a mechanism for the action of *vir* in females and males.

S12-16

A MALE-SPECIFIC GERMLINE MARKER: A TOOL TO STUDY GERMLINE SEX-DETERMINATION IN DROSOPHILA.

Monica Steinmann-Zwicky and Susanne Staab
Zoological Institute,University of Zurich,
Winterthurerstrasse 190,8057 Zurich,Switzerland

In *Drosophila*, germ cells already display sexual dimorphism in embryos. To find genes that are involved in early germline sex-determination, we have screened enhancer-trap lines for specific expression in the early gonads. One line is expressed in stem cells of the male germline. We detect its product already in embryonic germ cells, it therefore reflects the expression of one of the earliest sex-specific germline genes. XX germ cells express the male-specific marker in masculinized XX flies. The expression of this marker is therefore controlled by induction in XX germ cells. XY germ cells express the marker in feminized XY flies: these cells must express the marker autonomously. Thus, autonomous and inductive signals determine the sex of germ cells already in embryos.

S12-17

OVEREXPRESSION OF EF-1α IN *DROSOPHILA*

Matthias Gasser, Ruedi Ackermann, Stephen C. Stearns, and Christine Brack, Zoology Institute and Dept. of Cellbiology, Biocenter, University of Basel

Elongation factor Ef-1α is a nucleotide binding protein that catalyzes the binding of aminoacyl-t-RNA to the ribosome. It has been proposed that the age-related decline in protein synthesis observed in *Drosophila melanogaster* may result from decreased transcription of the EF-1α gene. Shepherd et al. found that transgenic *Drosophila melanogaster* carrying an additional copy of the Ef-1α gene under control of a heat inducible promotor showed an extended lifespan at 29°C relative to control flies. Ef-1α mRNA expression in Shepherd's lines has been quantitated by Shikama et al. They showed that transgenic Ef-1α flies did not have higher levels of EF-1α mRNA, EF-1α protein, and EF-1α activity at 29°C than control flies kept under the same experimental conditions. The transgene could be induced when transgenic flies were heat shocked at 37°C but could not be detected when the flies were kept at 29°C. Starting from Shepherd's original line Stearns et al. constucted six new lines with an additional copy of the EF-1α gene at different positions on the third chromosome. To test Shepherd's hypothesis they looked at effects of the EF-1α overexpression on lifespan as well as on other fitness parameters. They found that genetic manipulation can significantly affect lifespan and other fitness parameters although there was no general positive correlation between EF-1α overexpression and lifespan. Stearns et al. did not measure the expression of the transgenic EF-1α mRNA. As no transgenic message could be detected in Shepherd's lines we were investigating the expression of the transgene in the newly created EF-lines.

S12-18

Construction of a Target Gene Shuttle Vector to Investigate Mutagenesis in *D. melanogaster*

M. Hersberger[a], K. Kirby[b], J.P. Phillips[b], Th. Koller[a] and R. M. Widmer[a]
[a]Institute of Cell Biology, ETH-Hönggerberg, CH 8093 Zürich
[b]Dept. of Molecular Biology & Genetics, University of Guelph, Ontario, Canada

We are presenting data on a transgenic mutation test system in *Drosophila melanogaster*. Transgenic *D. melanogaster* were constructed, which were transformed with a shuttle vector, containing the bacterial *lacZ* gene as a target for mutagenesis. By restrictionendonuclease treatment of genomic DNA followed by ligation of the recircularized shuttle vectors, thousands of *lacZ* gene copies from somatic or germline cells can be rescued from one fly. The shuttle vectors can then be transformed back into *E. coli lacZ*⁻ mutants, where the activity of the *lacZ* genes can be scored. The number of inactivated versus intact *lacZ* genes directly indicates the mutagenic activity of a chemical. Results with ethyl-nitroso-urea (ENU) as a direct acting mutagen show an increase in frequency of the inactivated *lacZ* gene in the ENU treated group compared to the control group.

S12-19

CHARACTERIZATION OF THE *WINGLESS*-RESPONSIVE ELEMENT OF THE *GOOSEBERRY* GENE.

Joy Alcedo and Markus Noll.
Institut für Molekularbiologie II, Universität Zürich, CH-8057 Zürich, Switzerland.

Promoter analysis of the segment-polarity gene *gooseberry* (*gsb*) in *D. melanogaster* showed that the activation and maintenance of the gene is controlled by two separate elements. Its striped expression is activated in each embryonic segment by pair-rule proteins at the late blastoderm stage via the *gsb*-early element (GEE) and maintained by the the segment-polarity protein Wingless (Wg) after germ band extension via the *gsb*-late element (GLE) (Li *et al.* 1993. *EMBO J.* 12, 1427-1436). Through sequence comparisons between the GLE of *D. melanogaster* and the promoter region of *gsb* in *D. virilis*, we defined a shorter region (200 bp) within the 739-bp GLE fragment, which requires the *wg* protein to activate the late expression of a *gsb-lacZ* fusion gene. The 200-bp activator element also contains a 60-bp sequence that specifically recognizes a 125 kD protein in nuclear extracts of *D. melanogaster* embryos in Southwestern blot analyses. In addition to an activator element, the 739-bp GLE contains a repressor element. We observed broader stripes of expression in transgenic lines with the *gsb-lacZ* fusion gene under the control of the 200 bp fragment than in lines with the *gsb-lacZ* fusion gene under the control of the 739-bp GLE, although the temporal expression pattern of the fusion gene is identical in all lines. We are using the 200-bp activator element to screen a *D. melanogaster* cDNA library to isolate the *wg*-responsive factor(s) by genetic selection in yeast.

S12-20

FUNCTIONAL ANALYSIS OF THE *DROSOPHILA* *gooseberry* AND *paired* PROTEINS

Lei Xue, Xuelin Li, and Markus Noll
Institut für Molekularbiologie II, Universität Zürich, CH-8057 Zürich, Switzerland

In the *Drosophila* genome, three genes performing distinct developmental functions have been found to encode transcription factors with a paired-domain and a *paired*-type homeodomain in their N-terminal halves. The pair-rule gene *prd* activates the segment-polarity genes *gsb, wg* and *en* in segmental stripes in the blastoderm, while *gsb* maintains the expression of *wg* at a later stage through a *wg-gsb* autoregulatory loop and also activates *gsbn* expression in the CNS. Previous work from this lab demonstrated that ubiquitous expression of *prd, gsb* or *gsbn* generates the same phenotype and, more surprisingly, that the transgene in which the *prd* coding region is under the control of the *gsb* cis-regulatory region is able to rescue the cuticular phenotype of *gsb* mutant embryos. These results suggest that these three proteins have retained the same functional potential despite the different functions of their genes and their highly diverged C-terminal halves. More recently, the following results, corroborating this hypothesis, were obtained: (i) rescue of *prd* mutants to adult by the *gsb* coding region under the control of the *prd* regulatory region; (ii) rescue of the cuticular phenotype of *prd* mutant embryos by the coding region of their mammalian homolog, *Pax-3*, under the control of the *prd* regulatory region; and (iii) none of the three truncated Gsb proteins, in which either the paired-domain or homeodomain or both have been deleted, under the control of the *prd* regulartory region, mimics the function of the Prd protein. It follows that both DNA binding domains, the paired-domain and the *paired*-type homeodomain, are necessary for the normal function of *prd*.

S12-21

TARGETED GENE EXPRESSION IN *DROSOPHILA* BY MEANS OF TETRACYCLINE-RESPONSIVE PROMOTERS

Bello Bruno and Walter J.Gehring, Dept. of Cell Biology, Biozentrum, University of Basel, Klingelbergstr. 70, CH-4056 Basel

We are developing a new method for targeting gene expression in a spatial and temporal restricted manner. We make use of the possibility to stimulate transcription of a promoter fused to the tet operator sequences upon binding of a fusion protein comprising the bacterial Tet repressor (TetR) and the activation domain of VP16 (Gossen, M. & Bujard, H. PNAS 89, 5547, 1992). Tetracycline prevents this binding by forming a stable complex with the tet repressor thus maintaining the promoter in a silent state. When transiently expressed in *Drosophila* cells, TetR-VP16 leads to a 500 fold activation of a TetO-LacZ reporter gene that can be inhibited with 1 µg/ml of tetracycline. We have now generated transgenic lines for targeting expression of the lacZ gene in the eye imaginal disc throughout the larval development. This test system allowed us to show that the lacZ gene can be kept in a silent state by feeding the larvae on a medium containing as little as 0.1 µg/ml of tetracycline and subsequently induced by shifting the larvae to a tetracycline-free medium.

S12-22

THE *DROSOPHILA* SRF HOMOLOG IS REQUIRED FOR FORMATION OF INTERVEIN TISSUE IN THE WING AND IS ALLELIC WITH *blistered*

Jacques Montagne*, Jay Groppe*, Karen Guillemin+, Mark A. Krasnow+, Markus Affolter* & Walter J. Gehring*
*Biozentrum, University of Basel, CH-4056 Basel,
+Stanford University School o Medicine, Stanford, CA 94305

In cell culture experiments, the Serum Response Factor (SRF) plays a key role in the activation of the c-*fos* promoter. To determine SRF functions in a whole organism, we have characterized molecularly and genetically the *Drosophila* homolog (DSRF). We have observed two loss-of-function phenotypes: (i) in homozygous mutant embryos, terminal tracheal cells fail to develop fine terminal extensions; (ii) in heterozygous adult flies, additional vein tissue develops in the wing (haplo-insufficient phenotype). Immunolocalization shows that DSRF is expressed in the future intervein tissue of the wing imaginal disc. Clonal analysis reveals that in absence of DSRF, intervein tissue fails to differentiate and is vein-like in appearance. The DSRF gene maps to the same chromosomal location as *blistered (bs)*, mutations which produce additional veins and blistered wings. Genetic analysis of wing phenotypes suggest that SRF and bs are allelic. We are currently analyzing various *bs* mutants for molecular lesions in the DSRF gene.

S12-23

CHARACTERIZATION OF *STAND STILL*, A *DROSOPHILA* GENE INVOLVED IN GERMLINE SEX DETERMINATION AND FEMALE GERM CELL SURVIVAL.

Pennetta, G. and Pauli, D. Département de Zoologie et Biologie Animale, Université de Genève, 154 Route de Malagnou, 1224 Chêne-Bougeries

Several genes have been shown to be required for the determination of the sexual identity of the germ cells in *Drosophila melanogaster*. We have characterized *stand still*, which is required in the female but not in the male germ line. Mutations in *stand still* cause two characteristic phenotypes. The strongest effect is the death of female germ cells. Surviving germ cells show a sexual transformation and differentiate as spermatocytes. We shall present our progress in the genetic and molecular characterization of this gene.

S12-24

REQUIREMENT OF THE *DROSOPHILA* GENE *NOISETTE* IN THE SURVIVAL OF THE MALE GERM CELLS.

Laget, V., Oliver, B. and Pauli, D. Département de Zoologie et Biologie Animale, Université de Genève, 154 Route de Malagnou, 1224 Chêne-Bougeries

One of the consequences of incorrect sex determination in the *Drosophila melanogaster* germline is the death of the germ cells. We are analyzing mutations showing sex-specific sterility due to lack of germ cells. We shall describe our analysis of a gene called *noisette*. A transposon-induced mutation causes the disappearance of the stem cells in male but not in female gonads. The few stem cells which survive do not differentiate further than mature spermatocytes prior to meiosis. This results in sterility of males, while females are fertile. Mobilization of the transposon allowed the recovery of additional alleles. We shall present our progress in the genetic and molecular charaterization of *noisette*.

Proteases in cell-cell and cell-matrix interactions

S13-01

THE CHARACTERIZATION OF THE MESANGIAL CELL MATRIX METALLOPROTEINASES

H.P. Marti[1], L. L. Lee[2], J. Turck[2] and D.H. Lovett[2] from the Division of Nephrology, Department of Medicine, [1]Inselspital Bern, Switzerland and the Department of Medicine, [2]San Francisco VAMC-University of California at San Francisco, USA.

The degradation of the glomerular extracellular matrix is dependent upon the action of matrix metalloproteinases (MMP). For the cloning of the MMP from mesangial cells (MC) we developed a PCR-based structural homology cloning technique. Using this method, we obtained the cDNA of human punctuated metalloproteinase (PUMP-1) and interstitial collagenase, as well as of rat 72-kda type IV collagenase. Steady state levels of the expression of 72-kda type IV collagenase and of PUMP-1 in cultured MC were greatly stimulated after incubation with IL-1β and TNFα. Following induction of acute anti-Thy 1.1 glomerulonephritis in rats, a significant increase in 72-kda type IV collagenase expression by proliferating MC was demonstrated. An antisense strategy utilizing an episomally-replicating pRBK vector was used for stable transfection of cultured mesangial cells to significantly reduce the constitutive expression of the 72-kda type IV collagenase. The transfected mesangial cells reverted from an inflammatory phenotype to the quiescent state characteristic of the normal glomerular mesangium. Therefore, MMP may represent a target of new forms of treatment of glomerulonephritis.

S13-02

HEPATOCYTE GROWTH FACTOR STIMULATES EXTENSIVE DEVELOPMENT OF BRANCHING DUCT-LIKE STRUCTURES BY CLONED MAMMARY GLAND EPITHELIAL CELLS.

J. V. Soriano, M. S. Pepper, T. Nakamura[1], L. Orci and R. Montesano. Department of Morphology, University of Geneva, Switzerland. [1]Division of Biochemistry, University of Osaka, Japan.

To investigate the mechanisms by which stromal-epithelial interactions influence mammary gland development, we assessed the effect of fibroblast-derived diffusible factors on the morphogenetic properties of a clone (TAC-2) of NMuMG mammary gland epithelial cells. In control conditions, TAC-2 cells suspended in collagen gels formed short cord-like structures. Addition of fibroblast conditioned medium dramatically stimulated cord formation and branching, resulting in the development of highly arborized duct-like tubular structures. The effect of conditioned medium was abolished by antibodies against hepatocyte growth factor/scatter factor (HGF). In addition, recombinant HGF (20 ng/ml) induced a 77-fold increase in mean cord length per colony. The effect of HGF was potentiated by hydrocortisone, which also enhanced lumen formation. These results suggest that HGF is an important stromal mediator of mammary gland development.

S13-03

N-Cadherin-catenin mediated cell contact in adult rat cardiomyocytes. Truncated N-cadherin mutant impair cell contact and communicattion

Cecilia Hertig, Monika Eppenberger, *Stefan Butz, *Rolf Kemler and Hans M. Eppenberger. Institute of Cell Biology, ETH Hönggerberg, CH-8093 Zürich
& *Max Planck Institut for Immunobiology, D-79011 Freiburg in Brsg.

Adult rat cardiomyocytes (ARC) in culture represent an ideal system to explore key questions of cell differentiation and cell contact. These cells do not undergo cell division and grow by cellular hypertrophy. It is assumed that extending pseudopodia establish cell-cell contact, subsequently, the intimate contact structure results in the formation of intercalated discs like structures (Eppenberger, H.M., et.al.,1994, TCM 4, 187-192). Adherens junctions form a particular part of the cell junctions where cadherin is concentrated. Cadherins are transmembrane glycoproteins that homophilically interact via the extracellular domain in a Ca2+ dependent way with neighbouring cells, while the cytoplasmic domain contains the catenin binding domain that mediates the interaction to cytoskeleton (Takeichi, M., 1991. Ann.Rev. Biochem. 59,237-252; Geiger, B. & Ayalon, O., 1992, Ann.Rev.Cell Biol. 8, 302-332; Kemler, R., 1993, TIGS, 9, 317-321).
We report here, that exogenous chicken N-cadherin is indeed expressed in ARC and specifically localized in the sites of contact. Cadherin overexpression does not disturb the precise localization, moreover, complements function of the endogenous homologue in the newly formed contact sites. To study the role of N-cadherin in ARC a dominant negative mutant was constructed to impair endogenous localization and function. It is assumed that coexpressed cadherin isoforms in the same cell would compete for catenin binding which are required for function (Kintner, C., 1992, Cell, 69,225-236). The disruption of the cadherin mediated cell contact led to a splitting of the whole intercalated disc with concomitant cell retraction. There is also evidence for the appearance and localization of two different cadherin-catenin complexes that may play a distinct role during the stabilization of the cadherin-mediated cell adhesion, the development of other cell interactions and the organization of the cytoskeleton.

S13-04

BLEB FORMATION, POLARITY AND LOCOMOTION OF WALKER CARCINOSARCOMA CELLS ARE SUPPRESSED IN HYPERTONIC MEDIA

Fedier A. and Keller H.U., *Institute of Pathology, University of Bern, CH-3010 Bern*

The available evidence suggests that the forward expansion of the cell membrane in locomoting blebbing cells can not be due to elongation of actin filaments. Therefore, the putative role of hydrostatic pressure as a primary force producing forward movement of membrane was investigated in Walker carcinosarcoma cells using hypertonic media. Under this experimental condition (0.2M sorbitol) blebbing, cell polarity and locomotion are completely suppressed. The average cell volume is reduced by 20%. The reduction of cell volume preceeds the inhibition of blebbing and locomotion. In contrast, the relative amount of F-actin is not significantly affected. F-actin staining occurs along the cell membrane in isotonic as well as in hypertonic media.
The findings are compatible with the hypothesis that blebbing, locomotion and cell polarity are suppressed, because the hydrostatic pressure is too low to act as a driving force.

S13-05

PKC SELECTIVELY REGULATES LYMPHOCYTE SHAPE, LOCOMOTION, PINOCYTOSIS AND F-ACTIN

S. Trachsel und H.U. Keller, Institute of Pathology, University of Bern, 3010 Bern, Switzerland

The role of protein kinase C (PKC) in lymphocyte functions (cell shape, locomotion, pinocytosis, F-actin levels) has been analysed using the PKC activator PMA and the highly selective PKC inhibitor Ro 31-8220. The highly selective PKC inhibitor Ro 31-8220 produces polarity and locomotion in initially resting B and T lymphocytes whereas the relative amount of F-actin is reduced. PMA antagonizes the polarizing effect of Ro 31-8220, suggesting that the spherical shape and the F-actin content of resting cells is controlled by constitutive PKC activity. Ro 31-8200 inhibits PMA-induce fluid pinocytosis, actin polymerization and development of nonpolar cells with surface.protrusions. Thus, lymphocytes can be stimulated to exhibit fluid pinocytosis using PMA and then activity can be switched to locomotion using Ro 31-8220. The results suggest that PKC plays a pivotal role in selectively switching lymphocyte function between resting state, locomotor activity and fluid pinocytosis. Ro 31-8220 produces an unusually high proportion of polarized and locomoting lymphocytes within a few minutes.

S13-06

THE SPECIFIC NH_2-TERMINAL SEQUENCE AC-EEED OF α-SMOOTH MUSCLE ACTIN PLAYS A ROLE IN POLYMERIZATION.

Chaponnier C., M. Goethals, F. Gabbiani, G. Gabbiani and J. Vandekerckhove. Department of Pathology, University of Geneva, Switzerland and Laboratory of Physiological Chemistry, State University of Ghent, Belgium.

Actin isoforms are characterized by slightly different sequences particularly at their NH_2-terminal ends. The specific anti-α-smooth muscle (SM) actin monoclonal antibody, anti-αSM-1, or its Fab fragment, provokes an increase of α-SM actin polymerization in vitro by decreasing the critical monomer concentration. The epitope recognized by the antibody has been shown to be located at the NH_2-terminal end of the SM actin molecule and to be restricted to the AcEEED sequence. This sequence appears also to be important in the filamentous organization of α-SM actin in vivo. Thirty minutes after microinjection of AcEEED into cultured aortic SM cells, one observes the selective disappearance of α-SM actin containing filaments while γ- and β-actin containing stress fibers remain unaffected. After microinjection, the biotinylated AcEEED locates along stress fibers with α-SM actin during the first 10 minutes. These results are consistent with an active turnover of α-SM actin into stress fibers and suggest that AcEEED traps a protein interacting with the NH_2-terminal sequence of α-SM actin and activating polymerization. (Supported by the Swiss National Science Foundation Grant # 31-40372.94 and 31-34062.92 and a grant from the Concerted Actions (OOA) of the Flemish Community)

S13-07

DIFFERENTIAL EXPRESSION OF A METALLOPROTEASE IN STRIATED MUSCLE TRANSDIFFERENTIATION OF JELLYFISH

Tair-Long Pan, Volker Schmid and Jürg Spring; Zoological Institute, University of Basel, Rheinsprung 9, CH-4051, Basel

Metalloproteases play important roles in normal development and pathological changes. Addition of proteases can alter the state of cellular differentiation in striated muscle from the marine jellyfish Podocoryne carnea. A cDNA clone coding for a novel metalloprotease of the astacin family was isolated. It contains a zinc-binding motif similar to the collagenase-type matrix metalloproteases. The rest of the astacin-type domain is different from the collagenase-type domain and occurs in many well studied systems: secreted as digestive enzyme in crayfish (astacin) or membrane-bound as a morphogenetic enzyme in Drosophilia (tolloid) and vertebrates (bone morphogenetic protein 1) and as an extracellular matrix degrading enzyme (meprin). The jellyfish enzyme appears to be secreted like astacin but is most similar to the membrane-bound meprin, suggesting that it represents a new subfamily of proteases or the common ancestor of astacin- and meprin-type enzymes. A probe of this clone detects a 1 kb mRNA on northern blots. The gene is differentially expressed during transdifferentiation as judged by PCR studies. The role of this enzyme in normal development and transdifferentiation will be analyzed more extensively.

S13-08

PURIFICATION FROM HUMAN PLASMA AND CHARACTERIZATION OF A PROTEASE CLEAVING VON WILLEBRAND FACTOR

Furlan M., Robles R. and Lämmle B. Hämatologisches Zentrallabor der Universität Bern, Inselspital, CH-3010 Bern.

Von Willebrand factor (vWF) mediates platelet adhesion to the subendothelium of the damaged vessel wall. vWF is synthesized in endothelial cells and secreted into plasma as multimers (MW>20'000 kD) composed of disulfide-linked repeating subunits. In the circulation, the hemostatically active high Mr vWF polymers are degraded to smaller inactive forms. We purified (>1000x) from human plasma a vWF degrading protease, using chelating Sepharose, ion exchange, hydrophobic interaction chromatography and gel filtration. High Mr vWF was incubated with the purified protease and the degraded material subjected to SDS-PAGE. The size and the amino acid sequence of the reduced fragments confirmed that the peptide bond 842 Tyr - 843 Met had been cleaved in vWF (the same bond that is assumed to be cleaved *in vivo*). Proteolytic activity had a pH optimum at 8-9 and was strongly inhibited by chelating agents (citrate, EDTA, EGTA), while only slow inhibition was noted with NEM. There was no inhibition by iodoacetamide, DFP, PMSF, TLCK, TPCK, SBTI, aprotinin, pepstatin and bestatin. The enzyme is not related to matrix metalloproteinases, since its activity is not dependent on the presence of zinc ions. Activation by metal ions was found to increase in the following order: Zn≈Cu≈Cd≈Ni≈Co≈Mn≈Mg<Ca<Sr<Ba. The observed properties of the vWF degrading enzyme differ from those of all other hitherto described proteases.

S13-09

REDUCED MIGRATION OF RAT LIVER EPITHELIAL CELLS GROWN ON COLLAGEN TYPE 1 CORRELATES WITH AN INCREASE OF PLASMINOGEN ACTIVATOR IN THE MEDIUM

Mayer-Jaekel, R.E., Feindler-Boeckh, S. and Bade, E.G.
Universität Konstanz, Postfach 5560, 78434 Konstanz

Normal rat liver epithelial cells can be induced to migrate by EGF treatment as shown by immunhistological detection of laminin in their migration tracks. Their Ha-Ras transformed derivatives migrate constitutively but can still be stimulated by EGF to maximal migration. The migration apparently correlates with the amount of proteases secreted into the culture medium. Culturing these cells on collagen type 1 coated dishes leads to a marked reduction in the migration, except for the EGF-induced migration of the Ha-Ras transformed cells. In this case only a change in the morphology but no reduction of migration can be observed. The extent of migration parallels the production of the plasminogen activator inhibitor type 1 as shown by immunoblotting and reverse fibrin zymography. The urokinase type plasminogen activator is secreted by migrating cells, but collagen type I apparently leads to a change in its localization: in the absence of collagen the enzyme is more associated with the cell layer, whereas it is detected predominantly in the medium of cultures on collagen-coated dishes, suggesting a release from the cell surface.

S13-10

MOLECULAR CLONING OF α-TUBULINS FROM *NEUROSPORA CRASSA*, TRANSCRIPTIONAL REGULATION, AND OVER EXPRESSION OF RECOMBINANT PROTEINS IN *E. COLI*.

Monnat, J., Turian, G., Lab. de Microbiologie générale, Sciences III, Université de Genève, 1211 Genève 4

In most eukaryotes the tubulin genes comprise small multigene families with approximately equal numbers of genes for α and β-tubulin, the structural proteins of microtubules...We have isolated a full cDNA of α-Tub B and a partial cDNA of α-Tub A. Southern blot analysis at low stringency has shown that there are only two genes coding for α-tubulin in *N. crassa*, whereas three isoforms of α-tubulin have been detected. We have shown that the level of Tub A and Tub B transcripts increases during the first six hours of conidial germination, then decreases gradually for the next ten hours. Tub A is not transcribed in dormant macroconidia and in the first thirty minutes of germination, whereas Tub B transcript is present during all the developmental course of *N. crassa*. We have overexpressed the fusion proteins in *E. coli*. The recombinant proteins are recovered in inclusion bodies. We are currently trying to purify α-Tub B to attempt to refold it, and we plan to induce the polymerization of the heterodimer with β-tubulin in protofilaments.

S13-11

COMPUTER - AUTOMATED DETECTION OF ELASTIN IN ELECTRON MICROGRAPHS

S. A. Tschanz, B. Haenni, P. M. Kalenga and P. H. Burri
Institute of Anatomy, University of Berne, CH 3012 Berne, Switzerland

In order to morphometrically analyze the variations of the elastin content in the lung parenchyma under various experimental conditions, we established a method allowing to detect and to quantify elastin in electron micrographs by means of computer image processing.

Lungs from malnourished and control rats were fixed and processed for morphometric analysis. First, using classical point counting methods on LM sections, we quantified the lung parenchyma. Ultrathin sections were triple stained with uranyl acetate, tannic acid and lead citrate. By these means, the elastin in the interalveolar septa appeared intensely stained in the EM. Using the NIKON Coolscan® film scanner, the electron micrographs were digitized and analyzed in a PC equipped with a MATROX IMAGE 1280® board. With VISILOG®, an image analysis software we applied a defined set of image processing algorithms, such as filtering, binarisation and morphological image operation, thus allowing the areal proportion of elastin per parenchyma (i. e. volume density), to be automatically detected by the computer. Knowing the amount of parenchyma in the lung, absolute elastin quantity can be calculated.

The advantage of this method over biochemical analysis is the direct and specific detection of elastin within the interalveolar septa with exclusion of the perivascular and peribronchial elastin.

S13-12

ADEQUATELY HIGH PRESSURE FROZEN EXTRACELLULAR CARTILAGE MATRIX EXHIBITS NO MESHWORK STRUCTURE

Daniel Studer, Martin Michel, Jeannine Wagner, Ernst B. Hunziker
M.E. Mueller Institute for Biomechanics, University of Berne, Murtenstr. 30, 3010 Berne

Conventional chemical fixation with glutaraldehyde, followed by osmiumtetroxide, extracts about 60% of the proteoglycans present in the ECM. Almost no extraction occurs when the proteoglycans are precipitated by cations e.g. ruthenium hexaamine trichloride. However the precipitates are rather large and high resolution imaging is impossible. We have evaluated the structure of the ECM using high pressure freezing. 200µm thick sections of rat growth plate cartilage and 150µm thick sections of bovine articular cartilage were excised in a bath of hexadecene. The sections were, under hexadecene, firstly punched (diameter 1.7mm) and then transferred to an aluminium sandwich (corresponding in size to the sample). These samples were high pressure frozen in a Leica EM HPF machine, freeze-substituted in acetone containing 2% OsO_4 (16h at -90°C; 16h at -60°C; 12h at -30°C), embedded in epon and poststained ultrathin sections were investigated in the EM. The ECM showed no meshwork when adequately frozen. In growth plate samples the meshwork free ECM is limited to a thin superficial layer, the inner part of the sample showing a meshwork which most likely was generated by ice crystal formation during freezing (growing ice crystals concentrate the solutes on their ridge thus forming a meshwork). The lower radial zone of the articular cartilage was fully vitrified and showed no segregation patterns at all.

S13-13

EGF Induces transient Association of PLC-γ1 with EGF-receptor and F-Actin at Membrane Ruffles

B.M. Humbel
Utrecht University, Dept Molecular Cell Biology, NL-3584 CH Utrecht The Netherlands.

Addition of EGF to A431 cells results in dramatic changes in cell morphology. The cells form ruffles accompanied with increased actin polymerization. Activation of the EGF-receptor tyrosine kinase by binding EGF leads also to phosphorylation and activation of PLC-γ1. We have investigated the localization of PLC-γ1 during cell activation by EGF. It is shown that addition of EGF to A431 cells leads to a translocation of PLC-γ1 from the cytosol to the membrane fraction. Interestingly, this relocation is directed to the membrane ruffles. PLC-γ1 co-localizes with the EGF-receptor and F-actin at the membrane ruffles as shown by immunolabelling. The membrane ruffles are also significantly enriched in phosphotyrosyl proeins. 10 minutes after stimulation the membrane ruffles disappear and also the co-localization of PLC-γ1 with EGF-receptor and F-actin. These results support the notion that EGF activation of A431 cells leads to the formation of a signalling complex of EGF-receptor, PLC-γ1 and F-actin which is primarily localized at membrane ruffles.

S13-14

Determination of intracompartmental sorting specificity by double epitope-tagging competition

Komiyama, M.; von Arx, P.; Perriard, E.; Soldati, T.; and Perriard, J.-C.
Institute for Cell Biology, ETH, 8093 Zürich.

Various combinations of two different cDNAs of myosin alkali light chain (LC) isoforms, which were differently tagged with epitopes either from VSV-G protein or from mT polyoma antigen, were co-expressed in cultured cardiomyocytes from adult and neonatal rat ventricles in order to compare the sorting specificity of each isoprotein to sarcomeres within the same cell. Expressed isoproteins were detected by means of anti-VSV and anti-mT antibodies and their sorting patterns were analyzed by confocal laser scanning microscopy. Expressed fast skeletal muscle types (LC1f and LC3f) were always localized at the A bands of myofibrils, while nonmuscle type (LC3nm) was distributed throughout the cytoplasm. The slow skeletal muscle type (LC1sa) showed a weak sarcomeric pattern if it was co-expressed with LC3nm, but it was distributed throughout the cytoplasm when it was expressed in combination with LC1f, LC3f or another slow skeletal muscle type LC1sb, which is identical to the ventricular muscle isoform. The LC1sb was localized at the A bands when it was co-expressed with LC3nm or LC1sa, while it was distributed to the cytoplasm if co-expressed with LC1f or LC3f. Thus, the sorting specificity of LC isoforms increases in order of LC3nm, LC1sa, LC1sb, LC1f and LC3f. Further, expression of chimeric cDNAs revealed that the middle part of each isoprotein is responsible for the isoform specific sorting pattern.

S13-15

Differences of Cytoskeletal Dynamics in Cultured Neonatal and Adult Rat Cardiomyocytes

B.M. Rothen-Rutishauser, J.M. Messerli, E. Perriard and J.C. Perriard
Institute for Cell Biology, ETH-Hönggerberg, CH-8093 Zürich

Neonatal and adult rat cardiomyocytes in culture undergo a sequence of morphological changes in the presence of serum. Initially neonatal rat cardiomyocytes (NRC) are round and after attachment to the substrate the cells begin to spread and myofibrils emerge. Adult rat cardiomyocytes (ARC) however start from a rod-shaped cell, round up and most myofibrils degenerate. The cells then flatten, grow in size and myofibrillar assembly occurs. Microfilaments and microtubules can be influenced by addition of drugs. Cytochalasin D (CD) binds to the barbed end of the actin filaments, nocodazole (NO) inhibits the addition of tubulin to microtubules and both result in filament depolymerization. NRC treated with CD are able to attach to the substrate but are totally inhibited from spreading. However, even if the microtubules in NRC are destroyed with NO the cells are able to attach and to spread but not to the same extend as control cells. ARC treated with CD or NO are completely inhibited from spreading but remain attached to the substrate. The drug treatment does not inhibit protein synthesis neither in NRC nor in ARC. While in treated NRC myofibrils survive, in ARC a complete degeneration is observed and the accumulation of myofibrillar proteins is decreased. Cell spreading and myofibrillogenesis in NRC appears to be more dependent on functional microfilaments whereas in ARC spreading is dependent on functional microfilaments and microtubules as well. In addition, ARC, inhibited from spreading, appear to have a degradation system which seems not to be present in NRC.

S13-16

IDENTIFICATION OF ACTIN IN FORAMINIFERA: PHYLOGENETIC PERSPECTIVES.

Fahrni J.F. and Pawlowski J.

Dpt. of Zoology and Animal Biology, University of Geneva, CH-1224 Geneva, Switzerland

Actin was identified by immunoblotting in 13 species of foraminifera representing four major taxonomic groups (Allogromiina, Textulariina, Rotaliina and Miliolina). Three actins of different molecular weight (MW) were detected. The MW of these actins (about 43, 45 and 46 kD) is larger than vertebrate skeletal actin and protistan cytoplasmic actin. Two actins of different MW were detected in Allogromiina, Textulariina and Rotaliina, while only one type was present in Miliolina. Differences between actins of distinct taxonomic groups of foraminifera suggest that actin might by an useful tool in the study of the molecular phylogeny of these microorganisms.

S13-17

GELATINASE INHIBITORS FOR THE TREATMENT OF CANCER

Andrew J.P. Docherty,
Celltech Therapeutics, Slough, SL1 4EN, UK.

The metastatic properties of cell lines transfected with representative members of each of the main classes of matrix metalloproteinase were compared in nude mice. Gelatinase-A but not stromelysin or collagenase was found to convey a metastatic phenotype on the transfected cells that was dependent on both the catalytic properties of the gelatinase, and on properties determined by its non-catalytic C-terminal domain. Knowing the specificity of the autoproteolytic cleavages that occur during metalloproteinase activation we were able to design highly potent (K_i's $<10^{-11}$M) and selective gelatinase inhibitors (typically 10000-fold over collagenase). These compounds inhibit the gelatinases found in the cytosols of human breast tumours, and when long acting orally active analogues are administered to mice injected with the gelatinase-A transfectants, or a syngeneic colorectal tumour cell line, they reverse the metastatic phenotype. Our further finding that they can delay both angiogenesis and the subcutaneous growth of human xenografts in nude mice points to their potential for treating cancer in man.

S13-18

Recapitulation of a Normal Cellular Program in early Invasive Breast Cancer

V. Djonov, H.J. Altermatt, A. Arnold, H. Gerber*, R.R. Friis and A.-C. Andres*
Laboratory for Clinical and Experimental Research, Tiefenaustrasse 120, CH-3004 Berne, * Institute of Pathology, Murtenstrasse 31, CH-3010 Berne

The identification and characterization of biological markers reflecting not the evidence of, but rather the potential for, neoplastic progression may provide the means to predict individual cancer risk and to devise targeted protocols for treatment and prevention of breast cancer. In view of identifying such markers we undertook a comparative analysis of normal invasive mammary epithelial growth occurring at puberty and neoplastic growth using the mouse as a model system. We analyzed cell proliferation and the expression of the metalloproteinase stromelysin-1 as a marker for tissue remodeling and of the extracellular matrix protein tenascin as a marker for cell migration. The study revealed striking parallels between the normal pubertal growth phase of the mammary gland and the development of invasive, metastasizing mouse mammary tumors, whereas less aggressive, non-metastasizing tumors exhibited distinct growth characteristics. The myoepithelial origin of proliferation and of stromelysin-1 expression was a hallmark of both normal growth at puberty and the development of aggressive tumors in the mouse. The investigation of neoplastic lesions in the human breast indicates that in human carcinogenesis the pubertal growth characteristics are recapitulated in the ductal carcinomas only and that the expression of the tissue remodeling enzyme stromelysin-1 may define early stages of invasive and potentially malignant growth.

S13-19

CELL MORPHOLOGY AND uPA GENE REGULATION

Nagamine Y, Frixen U, Lee JS and Irigoyen J.-P.
Friedrich Miescher-Institut, PO Box 2543, CH-4002 Basel

Expression of urokinase-type plasminogen (uPA) is often observed in cells that exhibit dynamic changes in cell morphology, suggesting a link between cytoskeletal reorganization (CSR) and uPA expression. Breast carcinoma T47D cells express on the cell surface E-cadherin, an epithelial cell specific cell-cell adhesion molecule. When these cells were treated with the anti-E-cadherin monoclonal antibody DECMA, the cells dissociated from each other and lost their epithelioid morphology, paralleled with a rise in the secretion of uPA and the invasiveness on artificial collagen matrices. To study the molecular mechanism of uPA gene regulation by CSR, we then employed a system in which a high uPA expression could be obtained by directly reorganizing cytoskeleton. We induced CSR in renal epithelial LLC-PK_1 cells using pharmacological agents colchicine and cytochalasin. Upon treatment with these agents uPA gene was induced independently of PKC and PKA. Detailed analyses show that the uPA gene is induced via a Ras-Raf-ERK pathway leading to the activation of c-Jun which interacts with an AP-1 site located at 2kb upstream of the transcription initiation site. AP-1 is a positive regulator of growth and oncogenesis, and CSR is an integral part of these processes. Our results provide a view how CSR and AP-1 could be coupled in these processes.

S13-20

VASCULAR ENDOTHELIAL GROWTH FACTOR MODULATES EXTRACELLULAR PROTEOLYTIC ACTIVITY IN ENDOTHELIAL CELLS

S.J. Mandriota, G. Seghezzi*, J.-D. Vassalli, R. Montesano, P. Mignatti* and M.S. Pepper
Dept. of Morphology, University Medical Center, Geneva, Switzerland; *Università di Pavia, Italy

Angiogenesis is an essential requirement for the growth of normal and neoplastic tissues. In this process, finely tuned proteolytic activity facilitates endothelial cell migration into surrounding tissues. Vascular Endothelial Growth Factor (VEGF), a secreted angiogenic factor and endothelial cell-specific mitogen, is a major regulator of both physiological and pathological angiogenesis. We report here that VEGF increases both urokinase- and tissue- type plasminogen activator (uPA, tPA) expression in endothelial cells. VEGF also increases the expression of the high affinity cell surface receptor for uPA (uPAR) in the same cells, thus localizing uPA activity to the cell surface. PA-inhibitor type 1 expression is also increased in VEGF-treated endothelial cells, suggesting that a balance between proteolytic enzymes and their inhibitors is required for normal capillary morphogenesis.

S13-21

PLASMINOGEN ACTIVATION IN PHYSIOLOGY AND PATHOLOGY

André-Pascal Sappino and Jean-Dominique Vassalli
Departments of Medicine and Morphology, Geneva Medical School

Plasminogen activators (PAs) and their inhibitors (PAIs) are considered to be key participants in the balance of proteolytic and antiproteolytic activities that regulates extracellular matrix (ECM) turnover. In addition to its established role in fibrinolysis homeostasis, plasminogen activation is known to be involved in a wide variety of physiological processes. For instance, numerous cell types endowed with invasive properties, such as trophoblastic cells, neural crest cells, monocytes/macrophages, and regenerating keratinocytes, synthesize and secrete PAs, supporting the contribution of plasmin in ECM degradation required by tissue remodeling. Furthermore, PAs can be produced by cells devoid of invasive properties, such as epithelial cells lining tubular structures in the kidney, the mammary gland and seminal vesicles, suggesting that plasmin may help maintaining tubular fluidity. Finally, the demonstration of PA production by neurons of the central nervous system raises the possibility that plasmin could also influence the biological activity of peptides acting like hormones, by converting, for instance, inactive precursors of growth factors or by allowing their release from the ECM. Similarly, a large body of evidence indicates that plasmin-mediated proteolytic balance is altered during pathological processes. For example, excessive plasmin production has been consistantly linked to tissue invasion and metastasis formation in animal and human neoplasia, while reduced plasmin formation due to increased PAI production has been associated to deleterious protein deposition in other disorders. In conclusion, the PA/plasmin system is thought to modulate interactions between cellular and extracellular compartments in a wide variety of conditions. The exploration of physiological conditions involving plasminogen activation should provide insights into our understanding of their potential participation in pathological processes.

S13-22

Modulation of the epithelial phenotype during tumorigenesis: An *in vivo/in vitro* comparison

Martin Oft, Janos Peli, Claude Rudaz, Hartmut Beug and Ernst Reichmann
Institut Suisse De Recherches Expérimentales Sur Le Cancer (ISREC)

We are aiming to understand how specific epithelial properties, such as cell polarity, membrane trafficking, cell-cell and cell-extracellular matrix adhesion are altered during tumorigenesis and how these alterations relate to cell invasiveness and metastasis. We have recently employed an experimental approach to turn the function of "nuclear messengers" such as c-Fos (and c-Jun) on and off at will in polarized mammary epithelial cells. For this, constructs encoding the c-fos (or c-jun gene), fused to the hormone-binding domain of the human estrogen receptor (resulting in c-FosER/c-JunER fusion proteins) were used. We showed that long-term activation (24-48 hours) of c-FosER caused the disruption of epithelial cell polarity which finally led to an irreversible epithelio-fibroblastoid cell conversion. In addition we expressed an activated ras oncogene (which is acting upstream of c-Fos) in the same mammary epithelial cells. Interestingly, these Ras-expressing cells were only moderately altered in phenotype when cultured on conventional cell culture plastic. However, when injected into mice they developed into rapidly growing tumors which homogeneously consisted of cells that had lost epithelial properties but had gained fibroblastoid characteristics. Furthermore, when cultured within reconstituted extracellular matrices the phenotype of Ras-transformed cells could be highly modulated, again ranging from polarized/epithelial to spindle-shaped/fibroblastoid. This modulation was very much dependent on serum growth factors and well correlated with the expression of certain metallo and serine proteinases.

Structure-function relationship in membrane proteins

S14-01

A new structure for ligand-gated channels

R. A. North

Glaxo Institute for Molecular Biology,
14 chemin des Aulx, 1228 Plan-les-Ouates, Geneva, Switzerland

ATP can mediate synaptic transmission by acting at P_{2X} receptors. P_{2X} receptors are ion channels directly gated by the binding of ATP and some analogs; the ionic pore selects for cations, and has significant calcium permeability. Structural predictions based on the nucleotide sequences of cloned cDNAs suggest that the protein has two hydrophobic segments sufficiently long to cross the membrane and these are separated by a large, cysteine-rich, presumably extracellular domain. A similar overall structure has been proposed for the mechanosensitive channels of *Caenorhabditis elegans* and the related rat amiloride-sensitive epithelial sodium channel. This contrasts with the superfamily of ion channels gated by extracellular ligands (nicotinic, 5-HT_3, $GABA_A$, glycine, and excitatory amino acids receptors) that probably have four transmembrane spans and a long extracellular N-terminal. The results of mutagenesis experiments which seek to determine the molecular architecture of the channel will be reviewed.

S14-02

Characterization of the Na,K-ATPase β2 subunit as a neural recognition molecule

M. Schachner, Neurobiology, ETH, Hönggerberg, CH 8093 Zürich

The adhesion molecule on glia (AMOG) was first described as a Ca^{++} independent neural recognition molecule that mediates neuron-to-astrocyte interaction in vitro and that it is functionally involved in the migration of granule cells along Bergmann glial fibres in cerebellar explant cultures[1]. Sequence analysis revealed AMOG to be a close homologue of the β1 subunit of Na,K-ATPase[2], and it was therefore designated β2. β2 is a recognition molecule by several operational criteria. These include: β2 containing liposomes bind to neuronal cell surfaces and neurite outgrowth is enhanced on β2 transfected fibroblasts. The β2 subunit can functionally associate with the catalytic α subunits. β2 null mutant mice[3] show enlarged ventricles, degenerating photoreceptor cells, and swelling and degeneration of astrocytic endfeet, leading to vacuoles adjoining capillaries of brain stem, thalamus, striatum and spinal cord. The animals die at 17-18 days after birth. The combined observations will be discussed in view of the fact that a recognition molecule can be part of an ion pump.

1. Antonicek et al. (1987) J. Cell Biol. 104, 1587
2. Gloor et al. (1990) J. Cell Biol. 110, 165
3. Magyar, Bartsch et al. (1994) J. Cell Biol. 127, 835

S14-03

THE ADRENERGIC RECEPTORS: MODEL SYSTEMS FOR THE STRUCTURE-FUNCTION RELATIONSHIP OF G PROTEIN COUPLED RECEPTORS.

Cotecchia S. - Institut de Pharmacologie et Toxicologie, Rue du Bugnon 27, 1005 Lausanne.

The adrenergic receptors (AR) are members of the large superfamily of the seven transmembrane domain receptors coupled to guanine nucleotide regulatory proteins (G proteins) which transmit the signal from the extracellular surface to the intracellular effectors. Whereas the bundle formed by the transmembrane domains forms the ligand binding site of the AR, their intracellular loops are implicated in G protein coupling and they are targets of regulatory proteins such as protein kinases. The work accomplished with chimeric adrenergic receptors indicated that the "selectivity" of G protein coupling resides in the N-terminal portion of the third intracellular loop (iL), in agreement with results obtained with chimeric muscarinic and muscarinic/adrenergic receptors. However, receptor-G protein coupling might involve different intracelluair regions forming a three-dimensional binding surface of the receptor for the G protein. G protein coupled receptors exists in an equilibrium between an "active" and "inactive" state. So far, very little is known about how binding of the extracellular signals is converted in receptor activation. It was discovered that point mutations in the C-terminal portion of the third iL resulted in ligand-independent (constitutive) activation of different AR subtypes. These results suggest that the C-terminal portion of the third iL plays an important role in the activation of the ARs. The discovery of the constitutively active AR mutants might help to further elucidate the molecular mechanisms involved in receptor activation. In addition, spontaneously occurring activating point mutations have been recently found in other G protein coupled receptors (including the receptors for TSH and LH) and might be involved in the pathogenesis of some endocrine diseases. Thus, despite their amino acid sequence divergences, different G protein coupled receptors share several functional as well as molecular features with the ARs. However, future structure-function studies will elucidate additional properties and molecular mechanisms which might be specific for different subfamilies of G protein coupled receptors.

S14-04

STRUCTURAL AND FUNCTIONAL MODELS K+ Channels

H.R. GUY and S.R. DURELL, LMMB, DCBDC, NCI, National Inst. of Health, Bethesda, Maryland, USA

We are using a hierarchical, iterative approach to develop models of voltage-gated channels and their homologs. Our initial models predicted the protein's transmembrane topology and postulated which protein segments were involved in functional mechanisms such as activation gating, inactivation gating, ion selectivity, and ligand binding. Numerous laboratories have used mutagenesis experiments to confirm or support most of these hypothesis for Na^+ and K^+ channels. Recently we have developed more precise models of the P segments that determine the pore's ion selectivity and that form the outer vestibule where toxins such as charybdotoxin bind. The first part of our current models of K^+ channel P segments is helical; however, the more highly conserved middleand latter portion that determine the selectivity has a less regular secondary structure. The K^+ binding sites are postulated to be formed by backbone carbonyl oxygens. We have also modeled how charybdotoxin binds in the outer vestibule of voltage-activated and Ca^{2+}-activated K^+ channels and how the 8 hydrophobic M1 and M2 helices surround the P segments in inward rectifying K^+ channels.

S14-05

SIDE CHAIN ACCESSIBILITIES IN THE PORE OF A K^+ CHANNEL PROBED BY SULFHYDRYL-REAGENTS OF DIFFERENT DIAMETER AND CHARGE AFTER CYSTEINE-SCANNING MUTAGENESIS.

R.D. Zühlke, H-J. Zhang, Y. Liu, L.L. Kürz and R.H. Joho, Dept. of Cell Biology & Neuroscience, UT Southwestern Medical Center, Dallas, TX 75235, USA.

The segment between S5 and S6 of voltage-gated K^+ channels has been implicated to form part of the ion conduction pathway. Little is known about the conformation of this region although various models have been proposed. To gain insight into the secondary structure of this region, we used cysteine-scanning mutagenesis and sulfhydryl-specific, membrane-impermeant reagents of different diameter and charge to probe side-chain accessibilities of mutated amino acids in the pore region of Kv2.1 (DRK1). We previously identified several positions that, when mutated to cysteine, showed current reduction after superfusion with the small Cd^{2+} ion as well as the larger 6x10 Å methanethiosulfonate-derivative MTSET. These results suggested that the side chains of K356C, P361C, I379C, Y380C, and K382C were directly accessible from the extracellular environment, presumably facing the aqueous lumen of the ion channel pore. Mutant I379C exhibited the largest reduction (>95%) of current amplitude, suggesting that position 379 may be in a narrow part of the pore. To explore the molecular dimensions at position 379 of Kv2.1, we have begun to study the effects of several-sulfhydryl-specific reagents of different diameter and charge. Coupling positively or negatively charged MTS-derivatives leads to similar current reduction, suggesting that steric hindrance but not electrostatic effects plays a major role at position 379. Currently, we are testing several SH-specific reagents of smaller diameter in an attempt to uncover additional positions in the deep part of the ion conduction pathway.

S14-06

WHAT MAKES α7 NEURONAL NICOTINIC ACh RECEPTORS INWARDLY RECTIFY?

Ian C. Forster, Physiologisches Institut, Universität Zürich, Ch-8057 Zürich.

Strong inward rectification is a ubiquitous feature of native neuronal nicotinic ACh receptors (nAChRs) yet the underlying mechanisms at the molecular level remain to be determined. To this end a model system was chosen using homomeric nAChRs reconstituted from chick brain α7 subunit and expressed in *Xenopus* oocytes. Site directed mutagenesis of intermediate ring residues located towards the cytosolic end of the M2 domain established that these residues are critical determinants of inward rectification. Furthermore, they were also shown to determine inward rectification for the "desensitized open" state, a finding in line with the stratified organisation hypothesis for the M2 domain. Data suggest that for wild type α7 nAChRs rectification is due in part to open channel block by intracellular free Mg^{2+} at a site within the transmembrane electric field, nearer the cytosol. The residual rectification under nominally Mg^{2+}-free recording conditions, whose origin remains to be identified, could not be attributed to intrinsic gating of the receptors. Supported by Swiss NF Grant 31.36552-92.

S14-07

A STUDY OF THE PATH FOR CA^{2+} TRANSLOCATION ACROSS THE HUMAN PLASMA MEMBRANE CA^{2+} ATPASE

Foletti D., Vellani, F., Guerini, D. and Carafoli, E., Institute of Biochemistry III, ETH Zentrum Zürich

The plasma membrane Ca^{2+}-ATPase (PMCA) and the sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) are closely related P-type pumps involved in the regulation of the intracellular Ca^{2+} concentration.
Mutagenesis work on the SERCA pump (MacLennan, D.H. (1990) Biophys. J., **58** (6) 1355-1365) has identified aminoacid residues potentially involved in the process of the Ca^{2+} translocation across the protein, and thus the membrane. They are located in putative transmembrane domain 4,5,6 and 8. Based on the sequence homology between the PMCA and the SERCA pumps several mutants of the PMCA protein have been prepared and successfully expressed in COS-7 cells. They are presently being tested for their ability to transport Ca^{2+}. The results will be compared to those on the SERCA pump.

S14-08

EXPRESSION OF THE PLASMA MEMBRANE Ca^{2+} ATPASE ISOFORM 1: PMCA1

B. Pan, D. Guerini and E. Carafoli,
Institute of Biochemistry, ETH, CH-8092 Zürich.

Early attempts to construct a PMCA1CI full-length clone had failed. A systematic analysis made clear that two regions were responsible for this behaviour: one was sensitive to the orientation of the cloned fragment, another was responsible for its low yield. Taking these properties into account it was possible to construct a recombinant baculovirus to express the PMCA1 pump: high levels of active protein could be obtained after infection of Sf9 cells. Comparison with the PMCA4 pump expressed in the same system revealed that the PMCA1 protein had much higher affinity to ATP. The purified recombinant proteins showed a similar calmodulin dependence of the ATPase activity, although the PMCA1CI seemed to bind calmodulin more strongly. Indications were obtained that the PMCA1CI pump was phosphorylated by the cAMP dependent kinase.

S14-09

HUMAN PLASMA MEMBRANE CALCIUM PUMP ISOFORM 4: EXPRESSION OF 3 ALTERNATIVELY SPLICED VARIANTS

Brigitta Seiz Preianò, Danilo Guerini and Ernesto Carafoli, Institute of Biochemisty III, ETH Zürich

The plasma membrane Ca^{2+}-ATPase (PMCA) is involved in the fine regulation of the calcium concentration in eukaryotic cells. The PMCA gene 4 (PMCA 4, a housekeeping gene) is alternatively spliced at 3 different sites. The splicing at site C, located in the middle of the calmodulin binding domain of the pump, generates two pump isoforms, 4CI and 4CII. Isoform PMCA 4CI is widely expressed in tissues, whereas PMCA 4CII is only found in neuronal and cardiac tissues. Splicing at site B leads to a protein PMCA 4BICI in which one putative transmembrane segment is missing. Splice site A has not been investigated in this contribution.
The three different isoforms has been expressed using the baculovirus-Sf9 cell system. All three isoforms were purified and characterised. Isoforms 4CII and 4CI were active, whereas isoform 4BICI showed only partial activity, i.e. it only formed a phosphoenzyme from inorganic phosphate. PMCA 4CI had a stronger affinity to calmodulin than PMCA 4II. The two isoforms also exhibited other minor differences in the catalytic cycle.

S14-10

Cloning of the cDNA of the frog heart sarcolemmal Na^{+}/Ca^{2+}-exchanger

Tomoko Iwata, Alexander Kraev and Ernesto Carafoli
Institute of Biochemistry, Swiss Federal Institute of Technology (ETH)
8092 Zurich, Switzerland

The cardiac sarcolemmal Na/Ca exchanger is essential for the regulation of the heart contraction/relaxation cycle. Na/Ca exchanger cDNA clones have been isolated from various tissues and species. Here we report the cloning of the Na/Ca exchanger cDNA in a lower vertebrate (frog) heart. The clone was found to be the product of the NCX1 gene, and its sequence was well conserved with respect to other species even if the divergence was greater than among different mammalian species. More differences were found in the intracellular loop, whereas the transmembrane domains were well conserved. In particular, an extra sequence of 9 amino acids was found in a clone at the site where the alternative splicing occurs in the intracellular loop in other species. Since the insertion of this domain did not change the reading frame, it seems likely that this stretch should be coded by a novel exon, so far not found in mammals. Interestingly, it completes a "Walker's A" nucleotide binding motif, which is not present in any other mammalian Na/Ca-exchangers reported so far. Since this portion of the intracellular loop is the site of various regulation aspects, it could be related to the peculiarities of exchanger regulation in the amphibian heart.

S14-11

The 70-kDa component of the heart sarcolemmal Na^{+}/Ca^{2+}-exchanger preparation is the C-terminal portion of the protein

Tomoko Iwata, Carmela Galli, Paola Dainese, Danilo Guerini and Ernesto Carafoli
Institute of Biochemistry, Swiss Federal Institute of Technology (ETH)
8092 Zürich, Switzerland

The cardiac sarcolemmal Na^{+}/Ca^{2+}-exchanger was expressed in COS-7 cells by the vaccinia virus system as a fusion protein with a poly-His tag at its C-terminus. Extracts of cells expressing the exchanger construct without the His-tag reacted with an antiserum against the C-terminal portion of the main intracellular loop of the exchanger: in agreement with the finding routinely made on heart sarcolemma and on plasma membrane of cells expressing the cardiac exchanger gene, three bands of about 160, 120, and 70 kDa were detected in Western blots. All three bands shifted to higher molecular masses when the construct with the His-tag was expressed, indicating that the three proteins had the same C-terminus. Thus, the 70-kDa protein, whose nature has always been obscure, appears to be a degradation product of one of the two larger proteins. N-terminal sequencing of partially purified exchanger preparations has identified the cleavage site(s) producing the 70-kDa protein in the 257-269 residue region of the exchanger molecule.

S14-12

Identification of a nucleoside phosphatase activity for the major GPI-anchored glycoprotein present on the pancreatic zymogen granule membrane.

Soriani, M. and Freiburghaus, A.U.
Pancreatitis Laboratory, University Hospital,
CH-8091 Zürich, Switzerland

GP-2 is the most abundant glycoprotein present on zymogen granule membranes (ZGM). GP-2 represents the 35-40% of the ZGM proteins. The molecular mass of this protein varies between 80-92kDa in different animal species and an isoelectric point (IEP) of 5.25 was reported in the rat. GP-2 has been demonstrated to be attached to the ectoleaflet of the ZGM by a GPI anchor. We have subjected CHAPS or Triton X-100 solubilized zymogen granule membrane proteins from pig pancreas to native two dimensional polyacrylamide gel electrophoresis. The major protein isolated was a GPI anchored glycoprotein with an IEP of 5.2, able to hydrolyse di- and triphosphate nucleotides. SDS two dimensional polyacrylamide gel electrophoresis showed at the same IEP the presence of a 92kDa glycoprotein. These findings strongly suggest that this protein is GP-2. We propose a role of GP-2 in the energy consuming process of intracellular sorting or targeting of zymogen granules and their fusion with the apical plasma membrane.

S14-13

UPTAKE OF GPI-PLD BY DIFFERENT CELL LINES

Hari T., Bütikofer P., Wiesmann U. and Brodbeck U.
Institute of Biochemistry and Molecular Biology, University of Bern, Bühlstrasse 28, CH-3012 Bern

Glycosylphosphatidylinositol anchor-hydrolyzing phospholipases (GPI-PL) have been described in a number of organisms. The only mammalian GPI-PL isolated so far is a GPI-specific phospholipase D (GPI-PLD) from serum. Since the enzyme does not seem to be active on membrane-bound GPI substrate, its physiological role remains unclear. In order to test the hypothesis that the enzyme may be taken up from blood and becomes membrane-active in an intracellular compartment, we studied the uptake of purified GPI-PLD into mouse neuroblastoma Nb2A cells, and human fibroblasts. We found that the amount of enzyme taken up by Nb2A cells increased in a concentration-dependent way until saturation was reached. The uptake of GPI-PLD by Nb2A cells at 37 °C occured linearly over a period of 96 h, and the enzyme seemed to be stable intracellularly. A similar uptake of GPI-PLD was seen in human fibroblasts, however after 48 h of incubation enzyme activity decreased indicating that GPI-PLD was degraded intracellularly. At 4 and 15 °C uptake of GPI-PLD by Nb2A cells was inhibited.

S14-14

EXPRESSION OF GPI-PLD IN COS-1 CELLS

Küng, M., Stadelmann, B. and Brodbeck, U.
Institute of Biochemistry and Molecular Biology, University of Berne, Bühlstrasse 28, CH-3012 Bern

A cDNA clone of a glycosyl-phosphatidylinositol-specific phospholipase D (GPI-PLD) has recently been isolated from bovine liver cDNA libraries. The translation product contains a signal peptide of 23 amino acids and the mature protein of 817 amino acids. Using an *in vitro* transcription and translation system we expressed a protein with an apparent molecular mass of 90.2 kDa whereas expression of the GPI-PLD cDNA in COS-1 cells resulted in a protein of 115 kDa that specifically hydrolyzed GPI-anchored proteins. In the COS cell experiments, the majority (>90%) of GPI-PLD activity was secreted into the culture supernatant while less than 10 % was associated with the cell pellet. Immunofluorescence of permeabilized cells and protein immunoblot analyses confirmed the presence of GPI-PLD in both the cell pellet and the medium. Expression of a N-terminal GPI-PLD fragment of about 320 amino acids showed no GPI-hydrolyzing activity despite the fact that it could be detected in the cell pellet by immunoblotting and immunofluorescence.

S14-15

MYCOBACTERIAL LIPOARABINOMANNANS INSERT INTO GPI-RICH PLASMA MEMBRANE DOMAINS OF HOST CELLS.

S. Ilangumaran, S. Arni, M. Poincelet, J.-M. Theler*, Nasir-ud-Din and D. C. Hoessli. Dépts de Pathologie et *Biochimie clinique, CMU, 1 rue Michel-Servet, 1211 Genève 4.

Mycobacterial lipoarabinomannans (LAMs) are major virulence factors in leprosy and tuberculosis. They consist of complex carbohydrate polymers of mannose and arabinose that are thought to be anchored in the mycobacterial envelope via a glycosylphosphatidylinositol (GPI) linked to an oligomannosyl residue. In vitro incubation of purified LAMs with monocytes-macrophages induces the production of IL-1, IL-6 and TNF.
In an effort to define their mode of interaction with host cells, we showed that LAMs integrate lymphocyte and monocyte plasma membranes directly, without a need for a liposome vehicle. This transfer to eukaryotic plasma membranes was (i) critically dependent on the presence of acyl groups in the LAM molecule, as the transfer was completely abrogated by mild alkali treatment of LAM and (ii) inhibitable by preincubation of cells with the GPI anchor of LAMs, suggesting a specific role for the LAM GPI hexamannosyl glycan. Moreover, LAM transfer occurred preferentially into plasma membrane vesicles enriched in endogenous murine GPI-anchored glycoproteins, suggesting that mycobacterial GPI-linked carbohydrates interact more efficiently with specific lipid components of GPI-rich plasma membrane domains.

S14-16

HOMO- AND HETEROTYPIC GAP JUNCTION CHANNELS

Bukauskas F.F., Weingart R.
Dept. Physiology, University of Bern, CH-3012 Bern

Experiments were performed on HeLa cells transfected with various mouse connexins, Cx (provided by K. Willecke, Bonn). Gap junction channels were induced by pairing cells which expressed identical or different connexins. The coupling conductances, g_j, were assessed at 37 °C using the dual voltage-clamp method. Formation of homotypic channels was observed in all cases. With potassium aspartate in the pipette solution, the open channel conductance, γ_j*(main state)* was about (in pS): 45 (Cx32), 50 (Cx31), 70 (Cx43), 140 (Cx26), 200 (Cx40), 400 (Cx37) and 400 pS (Cx45). All channels underwent fast transitions between γ_j*(main state)* and γ_j*(residual state)* which were controlled by the transjunctional voltage gradient, V_j. The ratios γ_j*(main state)*/γ_j*(residual state)* ranged from 1/4 to 1/6. Formation of heterotypic channels was pronounced in case of Cx26-Cx32, Cx26-Cx45, Cx32-Cx37, Cx32-Cx45 and Cx37-Cx40 (g_j = 2 - 20 nS, i.e. comparable to homotypic cell pairs), moderate in case of Cx32-Cx40, Cx32-Cx43 (g_j = 0.1 - 1 nS) and weak in case of Cx26-Cx31, Cx26-Cx40, Cx31-Cx32 and Cx32-parental Cx (comparable to parental cells: 70% with no coupling, 30% with weak coupling, g_j = 0.04 - 0.2 nS). Cx26-Cx32, Cx32-Cx37 and Cx32-Cx45 channels showed a V_j-dependent γ_j*(main state)*. It increased linearly with increasing negativity at the cytoplasmic aspect of the Cx32 connexon. The slope was dependent on the composition of the pipette solution. V_j-dependent channel gating was present only when the cytoplasmic aspect of the Cx32 connexon was negative. In Cx26-Cx45 channels, the open channel probability, P_o, was dependent on the polarity of V_j; at V_j = 0 mV, P_o was < 1. The single channel data provide satisfactory explanations for the asymmetric relationships $g_j = f(V_j)$ at the macroscopic level.

S14-17

REGULATION OF GAP JUNCTIONS BY LIPOPHILIC AGENTS AND IONS SUPPORTS THE CONCEPT OF TWO GATING MECHANISMS

Weingart R., Bukauskas F.F.
Dept. Physiology, University of Bern, CH-3012 Bern

Experiments were performed on preformed pairs and induced pairs of insect cells (clone C6/36) with the dual voltage-clamp method. In these cells, the junctional conductance, g_j, depends on the junctional voltage gradient, V_j (function $g_j = f(V_j)$ is bell-shaped), and the membrane potential, V_m (function $g_j = f(V_m)$ is sigmoidal; g_j = 0 at positive V_m; Bukauskas & Weingart, Biophys J 67:613, 1944). We found that long chain alkanols (C_6-OH to C_{10}-OH) provoke a shift of the g_j/V_m relationship along the V_m axis. Control: $V_{m,o}$ = 10 mV; intervention: $V_{m,o}$ = -20 mV to -100 mV ($V_{m,o}$: V_m at which g_j is half maximal). The shift develops with time, is dose-dependent and fully reversible. The decrease in g_j caused by alkanols was restored by hyperpolarization of V_m. Exposure to arachidonic acid yielded similar results. Modification of pH_i also produced a shift of the g_j/V_m relationship. pH_i = 7-9: $V_{m,o}$ = 10 mV; pH_i = 6-5.5: $V_{m,o}$ = -75 mV; pH_i = 7-6: sigmoidal change in $V_{m,o}$ from 10 mV to -75 mV. Hyperpolarization of V_m led to a recovery of g_j. Comparable results were obtained upon decreasing pCa_i. At the single channel level, exposure to alkanols revealed slow transitions between the main state or residual state and the closed state and thereby stopped fast channel activity. Hyperpolarization of V_m or washout of alkanols yielded slow transitions in the opposite direction and hence reestablished fast channel activity. This suggests that chemical modulation of g_j involves V_m-gating, not V_j-gating. In pairs of neonatal rat heart cells, exposure to alkanols also revealed slow transitions between the main state or residual state and the closed state. This implies that their gap junctions possess, in addition to V_j-gating, a chemical gating similar to that seen in insect cells.

S14-18

NEONATAL RAT HEART CELLS: ELECTRICAL GATING OF GAP JUNCTION CHANNELS

Valiunas V., Bukauskas F.F., Weingart R.
Dept. Physiology, University of Bern, CH-3012 Bern

Pairs of neonatal rat hearts cells were used to study the gating properties of single gap junction channels. A dual voltage-clamp method allowed to control the voltage gradient between the cells (V_j) and measure the intercellular current flow (I_j). Two types of preparation were used: a) induced cell pairs (Bukauskas & Weingart, Biophys J **67**:613, 1994) and b) preformed cell pairs treated with uncoupling agents (75 µM SKF-525A, 3 mM heptanol or 100 µM arachidonic acid. In case of a), the first opening of a newly formed channel was slow (20 - 65 ms) and occurred 7 - 25 min after cell-to-cell contact. The rate of channel insertion was 1.4 channels/min. Associated with V_j pulses, the channels revealed two stable conductances, a main state, γ_j*(main state)*, and a residual state, γ_j*(residual state)*. a) and b) yielded similar results, hence the γ_j values were pooled. The fit of the data to the Gaussian revealed a peak for each conductance state. Values of γ_j were dependent on the pipette solution used. Cs-aspartate: γ_j*(main state)* = 60 pS, γ_j*(residual state)* = 12 pS; KCl: γ_j*(main state)* = 95 pS, γ_j*(residual state)* = 22 pS. The respective ratio γ_j*(main state)*/γ_j*(residual state)* was 5.1 and 4.3. This suggests that γ_j*(main state)* and γ_j*(residual state)* exhibit different ion selectivities. Transitions of I_j between the closed state and main state or residual state were slow (15 - 45 ms), transitions between the residual state and main state were fast (< 2 ms).

S14-19

FAST AND SLOW CURRENT TRANSITIONS IN GAP JUNCTION CHANNELS OF CONNEXIN43

Banach K., Meda P., Weingart R.
Dept. Physiology, University of Bern, CH-3012 Bern

The dual voltage-clamp method was used to study the properties of single gap junction channels of a communication deficient rat islet tumour cell line stably transfected with rat Cx43. In pairs of parental cells, no signs of intrinsic gap junctions were found by electrophysiology and immunohistology. In strongly coupled pairs of transfected cells, $g_j(inst)$ averaged 9 nS (g_j: gap junction conductance; *inst*: instantaneous) and $g_j(ss)$ revealed a bell-shaped dependency on V_j ($V_{j,o}$ = ±60 mV; *ss*: steady state; $V_{j,o}$: V_j at which g_j is half maximal). In the following studies, we used weakly coupled pairs with few gap junction channels. In these preparations, the analysis of single channel currents revealed two different types of open states: 1) a main state with a conductance of ~90 pS and insensitive to V_j (range tested: ±100 mV); 2) a residual state with a conductance of ~20 pS. Current transitions involving the main state usually were fast (<2 ms), those involving the residual state were slow (10 - 15 ms). Slow transitions were found mainly when V_j was large. Under this condition, i.e. V_j > ±80 mV, the incidence of slow transitions seemed to increase with V_j. Concomitantly, the channel open time and open probability decreased towards zero. The slow events observed under this condition resemble those seen in preparations with a single gap junction channel. Conceivably, they may be related to the insertion of new channels/removal of old channels. Alternatively, they may reflect the operation of a gating mechanism different from that which controls fast channel operation.

S14-20

HETEROLOGOUS EXPRESSION OF HUMAN AND MOUSE SECRETORY COMPONENTS

S. Cottet and B. Corthésy
Institut de Biologie animale de l'Université de Lausanne

The secretory IgA antibodies involved in protection of mucosal surfaces are constituted of dimeric IgAs and secretory component (SC). SC protein was produced in human HeLa S3 or in monkey CV-1 cells using the vaccinia/T7 RNA polymerase hybrid system or the classical vaccinia expression system. Our data show that there is no difference in human SC expression between the classical and the hybrid expression systems. Therefore, RNA accumulation and protein synthesis within the cell was assayed by northern blot and immunofluorescence, respectively. Moreover, mouse SC produced using the vaccinia/T7 RNA polymerase hybrid system, was purified by Concanavalin A-agarose affinity chromatography in order to assess in vitro reassociation with murine as well as human dimeric IgAs from hybridomas. The purification procedure provides an enrichment of mouse SC under native conditions, and the purified recombinant protein specifically binds murine as well as human dimeric IgAs.

S14-21

BIOCHEMICAL AND MOLECULAR STUDIES ON *GIARDIA* VARIANT SURFACE PROTEINS

Papanastasiou P., Bruderer T., McConville M., Li Y., Köhler P.
Institute of Parasitology, University of Zürich

The major surface proteins (VSPs) of *Giardia* trophozoites comprise a group of novel polypeptides that are cysteine-rich and can vary considerably both in sequence and size. Sequence analysis of the genes coding for two VSPs (CRISP-90 and CRISP-75), predicted a cysteine-rich polypeptide with features common to other giardial VSPs, including the presence of a large number of the tetrapeptide motif CXXC in the variable N-terminal region and a homologous hydrophobic C-terminal domain. Preliminary data on the genomic organisation of both genes in their active and silenced status have been obtained. The compositional analysis of purified CRISP-90 indicated that it contains short oligosaccharides made up of glucose and N-acetylglucosamine, probably attached via O-linkages to the polypeptide backbone. Additionally, the sulfhydryl groups of all of the cysteins of this purified VSP are blocked or at least non-reactive. CRISP-90 bound to nitrocellulose was found capable of binding zinc but further studies on the native protein have failed so far to confirm this result.

S14-22

CONSTRUCTION OF A FUSION PROTEIN: FROM ENZYME COMPLEX TO COMPLEX ENZYME

Mao, Q., Schunk, T., Gerber, B. and Erni, B., Institute for Biochemistry, University of Berne, CH-3012 Bern, Switzerland

The bacterial phosphotransferase system(PTS) comprises a group of proteins which can be grouped into functional units according to their position and function in the phosphotransfer cascade. Homologous functional units of different sugar-specific phosphotransferase systems are expressed sometimes as single polypeptides and sometimes as domains of a larger polypeptide. Three types of linker sequence have been observed in the interdomain regions: Ala-Pro linkers, LKTPGRED linkers and Q linkers. To address the questions why homologous functional units sometimes are fused and sometimes separated and whether linker sequences are of general use for the construction of multidomain proteins, a multidomain protein was constructed by fusion of the transmembrane $IICB^{Glc}$ subunit and the three cytoplasmic proteins, IIA^{Glc}, HPr and enzyme I of the bacterial phosphotransferase system. The subunits were linked in the above order with Ala-Pro rich hinges and the fusion protein overexpressed in *E. coli* and purified. The fusion protein is active *in vivo* and *in vitro*. The biochemical characterization will be discussed.

S14-23

A monoclonal antibody which inhibits acetylcholine release in Torpedo synaptosomes is able to bind acetylcholinesterase activity.

Eder-Colli, L., Roulet, E.*, Medilanski, J. and Dunant, Y.*.*
Department of Pharmacology, CMU, 1211 Geneva 4

A monoclonal antibody (8/38A) developped against *Torpedo* synaptosomal plasma membranes was found to inhibit acetylcholine (ACh) release in synaptosomes isolated from the electric organ of *Torpedo* (Eder-Colli et al., J. Neurochem. 53, 1419, 1989). On Western blots of SDS-PAGE containing *Torpedo* synaptosomal plasma membranes the pattern of polypeptides labelled by the antibody was identical to the one exhibited by a well-characterized anti-acetylcholinesterase (AChE) monoclonal antibody (gift from Dr. J. Massoulié). However, the anti-AChE antibody did not significantly affect ACh release. Also, antibody 8/38A coupled to protein A sepharose was unable to immunoprecipitate AChE activity. Immunoblot analysis of synaptosomes treated with proteinase K which solubilized a large proportion of membrane-bound AChE, showed no significant decrease in the labeling of the antigen. These data argued against AChE being the antigen recognized by the antibody 8/38A. Nonetheless, more recent experiments such as immunoblot analysis of AChE that had been immunoprecipitated by an anti-AChE antibody and sedimentation analysis of AChE solubilized from synaptosomal plasma membranes that were previously incubated with antibody 8/38A, strongly suggested that this antibody is recognizing AChE activity. It is now requested to investigate a possible involvement of AChE in ACh release.

S14-24

STRUCTURE-FUNCTION RELATIONSHIPS OF MITOCHONDRIAL MONOAMINE OXIDASE A AND B CHIMAERIC FORMS

J.Gottowik, P.Malherbe, G.Lang, M.DaPrada & A.Cesura
Pharma CNS, F. Hoffmann-La Roche Ltd, Basel.

In this work, we studied the structure- function relationships of monoamine oxidases (MAO) -A and -B by constructing chimaeric forms of the isoenzymes. After transient expression, the properties were studied using selective and nonselective substrates and inhibitors. The data suggested the existence of two sequences at the N-terminus putatively involved in the binding site of MAO-B. Conversely, the catalytic properties of MAO-A appeared to be relative insensitive to substitution of the N- and C-termini with the MAO-B sequences. Modification of the central 282 amino acid sequence of MAO-A with MAO-B was incompatible with activity. None of the engineered chimaeras showed a shift of their specificity from one MAO activity to the other.

S14-25

CLONING OF THE RAT UTROPHIN AND CHARACTERIZATION OF AN N-TERMINAL ISOFORM

Zuellig, R.A., Blake, D.J.*, Tinsley, J.* and Davies, K.E*.
Institute of Pharmacology, University of Zürich,
CH-8057 Zürich, Switzerland; and
*Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, England

Utrophin is an autosomal homologue of dystrophin. The dystrophin gene is located on the X-chromosome. Its product dystrophin is missing in muscle fibres of patients with Duchenne muscular dystrophy. The human utrophin was cloned recently and shows a significant similarity to dystrophin. It has a similar structure with 4 domains, the actin binding domain, the rod domain, the cysteine rich (CR) domain and the C-terminal (CT) domain. The actin binding and the CR-CT domains exhibit 85% amino acid sequence similarity, and with 60% sequence similarity the rod is less conserved.

A rat cDNA library was screened with cDNA fragments from mouse utrophin. Several positive clones were found and characterized. 3 clones were found to overlap and to cover the whole coding region for utrophin. The combined cDNA contains an open reading frame of 10'254 bp and encodes a protein of 3'419 amino acids. The rat utrophin exhibits a high similarity of 91% to the human utrophin.

The beginning (1.8 kb) of 2 further clones are identical to the 5'-utrophin sequence while their ends (0.3 and 0.1 kb) are identical among themselves but different from the rat utrophin. Northern blots and results from PCR reactions support the notion that these 2 clones represent a short alternative transcript of the rat utrophin.

S14-26

Coiled-coil regions in the exoplasmic portion of the human asialoglycoprotein receptor.

Marc D. Bider and Martin Spiess
Dep. of Biochemistry, Biozentrum, University of Basel, 4056 Basel

Oligomerization of proteins from individual subunits has been implicated as part of a quality control system which retains incompletely assembled proteins within the endoplasmic reticulum. One mechanism of oligomerization is the formation of α helical coiled-coils, which mediate specific interactions between different subunits in a stoichiometrically defined manner. On the amino acid level a coiled-coil region is characterized by heptad repeats, a sequence with a characteristic distribution of apolar residues in position a and d.

We are studying the structure of the human asialoglycoprotein receptor. This receptor is a heterooligomeric complex composed of two subunits, H1 and H2. Both subunits are type II single-spanning transmembrane glycoproteins with a short cytoplasmic domain, a transmembrane domain, and a larger exoplasmic domain. The exoplasmic domain contains two subdomains, the carbohydrate recognition domain and the stalk domain. In both subunits this stalk region is composed of a stretch of 10 heptad repeats interrupted twice by a short stagger of 4 amino acids. To study the formation of coiled-coils and to define the stoichiometry of these complexes, the stalk domains of both subunits have been expressed in E. coli and purified by the use of a His-tag. The helical character of the stalk domains is studied by analysis of CD spectra of both subunits alone and of a mixture of both subunits. Analytical ultracentrifugation, reoxidation studies as well as cross-linkers are used to analyse the stoichiometry of the formed complexes. Initial CD experiments for the H1 stalk provide experimental evidence for α helical coiled-coils.

S14-27

Topology of the *Escherichia coli* Mannose Transporter

Huber, F. and Erni, B., Institute for Biochemistry, University of Bern, CH-3012 Bern, Switzerland

The mannose transporter of the *Escherichia coli* phosphotransferase system transports and concomitantly phosphorylates mannose and related hexoses. It consists of a soluble $IIAB^{man}$ subunit and two membrane bound subunits IIC^{man} and IID^{man}. Protein fusions between IIC^{man} and IID^{man} and alkaline phosphatase (PhoA) were analyzed to determine the membrane topology of the $IICD^{man}$ complex. The fusions were generated by progressively deleting the gene *manYZ* from its 3' end and ligating the truncated gene to the '*phoA* lacking promoter and leader sequences. Three clusters of active PhoA fusions occur in the *manY* gene and one cluster in the *manZ* gene. Compared with the hydropathy plots of IIC^{man} and IID^{man} we suggest 5 membrane spanning helices in IIC^{man} and one in IID^{man}.

S14-28

THE PRO REGION OF HUMAN INTESTINAL LACTASE-PHLORIZIN HYDROLASE

Hassan Y. Naim, R. Jacob, Hussein .Y. Naim, J. Sambrook, M.J. Gething, Institute of Microbiology, University of Düsseldorf, Germany and University of Texas, Southwestern Medical Center, Dallas, USA

Human small intestinal lactase-phlorizin hydrolase (LPH) is synthesized as a single chain polypeptide precursor, pre-pro-LPH, that undergoes two sequential cleavage steps: the first in the endoplasmic reticulum (ER) to pro-LPH (215-kDa) and the second, following terminal glycosylation in the Golgi apparatus, to mature 160-kDa LPH (denoted LPHß). The LPHß molecule is subsequently targetted to the brush border membrane. Characterization of the N-terminal profragment (denoted LPHα) of pro-LPH using an epitope-specific, anti-peptide polyclonal antibody reveals that LPHα (i) has an apparent molecular weight of ~100 kDa, (ii) is not associated with LPHß after cleavage of pro-LPH has occurred, and (iii) is not transported to the cell surface or secreted into the extracellular medium. In biosynthetic labeling experiments a clear precursor/product relationship could be demonstrated between pro-LPH and the LPHα and LPHß polypeptides. Further, LPHα has a significantly shorter half life than LPHß. LPHα is neither N- nor O-glycosylated, despite the presence of 5 potential N-glycosylation sites. LPHα, which is rich in cysteine and hydrophobic amino acid residues, may fold rapidly into a tight and rigid globular domain in which carbohydrate attachment sites are no longer accessible to glycosyltransferases. When expressd independently in COS-1 cells, the LPHß polypeptide forms a misfolded, transport-incompetent molecule. We propose a role for the LPHα domain within the pro-LPH molecule as an intramolecular chaperone during folding in the ER.

S14-29

OBSERVATIONS ON THE HOMOPHILIC INTERACTIONS OF THE NEURAL CELL ADHESION MOLECULE L1

Holm, J., Appel, F. and Schachner, M., Department of Neurobiology, Swiss Federal Institute of Technology, Hönggerberg, CH-8093 Zürich

The neural cell adhesion molecule L1 is a multidomain protein that plays important roles in cell adhesion, migration and neurite outgrowth. It can interact with itself by a self-binding, i.e. homophilic adhesion mechanism. To determine the domains of L1 involved in homophilic binding, we have generated protein fragments of L1 in a prokaryotic expression system and used these covalently coupled to fluorescent microspheres to quantify aggregation between them by cytofluorometric analysis. Protein fragments containing the first and second Ig-like domains and the third fibronectin type III homologous repeat showed avid self-binding. Ig-like domains III and IV also showed some self-binding, whereas Ig-like domains V and VI and fibronectin type III homologous repeats 1 and 2 as well as 4 and 5 were less or not active. Binding between different domains was also observed: Fibronectin type III homologous repeats 4 and 5 interacted with Ig-like domains I and II, and fibronectin type III homologous repeats 3 to 5 with all Ig-like domains. Binding of L1 to itself was interfered with by all protein fragments tested, suggesting that also less avidly binding domains of L1 contribute to homophilic binding. These observations indicate prominent functional roles of both Ig-like domains and fibronectin type III homologous repeats in homophilic binding of L1.

S14-30

Investigations on the Function of the Peripheral Myelin Protein PMP22

Roland Naef, Sangeeta Pareek# & Ueli Suter
Swiss Federal Institute of Technology, ETH Hönggerberg Zürich
#Mc Gill University, Montreal, Canada

The peripheral myelin protein 22 (PMP22) is a hydrophobic glycoprotein of 160 amino acids with four putative transmembrane domains which is highly expressed in compact peripheral myelin. PMP22 is also expressed, however, in various non-neural tissues and is upregulated in growth-arrested fibroblasts in vitro. PMP22 has been suggested to have functions in cell adhesion, cell-cell communication and growth regulation.

In an attempt to elucidate these hypothetical functions, we have analyzed endogenous PMP22 expression in cultured Schwann cells and growth-arrested fibroblasts. In addition we have overexpressed PMP22 in transiently and stably transfected cell lines. In these transfectants, the PMP22 cDNA was placed under the control of the CMV promoter and stable cell lines were generated. PMP22 expression was monitored by northern blot analysis, immunocyto-chemistry and immunoprecipitation.

Comparative immunofluoresence studies and endoglycosidase H sensitivity showed that PMP22 is predominantly found in the endoplasmic reticulum (ER) of these cells. Interestingly, stable cell lines lost PMP22 expression after only a few passages.

Thus, a tetracycline-inducible expression system was used to tightly regulate PMP22 expression. Several HeLa cell lines with stable and inducible PMP22 expression were isolated. The consequences of PMP22 overexpression on growth and adhesion properties of these cell lines will be discussed.

S14-31

STRUCTURE/FUNCTION STUDIES OF THE MEASLES VIRUS RECEPTOR CD46

C.J. Buchholz, U. Schneider, T. Cathomen, and R. Cattaneo
Institut für Molekularbiologie I, Universität Zürich, Switzerland

The human cell surface glycoprotein CD46, an inhibitor of complement activation, is also the measles virus (MV) receptor. The extracellular part of CD46 is always composed of four short consensus repeats (SCR's), each about 60 amino acids in length. CD46 has four major isoforms, generated by alternative splicing, which bear either one or two copies of a ser/thr/pro-rich region and one of two alternative cytoplasmic tails.

We are interested in the MV receptor function of CD46. Complete CD46 cDNAs were cloned from the brains of three patients who died with lethal persistent MV infections and from a control brain. From these brains we obtained only cDNAs coding for one of the two cytoplasmic tails, whereas from HeLa cells cDNAs coding for all four major isoforms were cloned. Most of the cDNA-encoded proteins mediated MV dependent cell fusion, indicating that functional MV receptors were present in all the brains examined, as expected. To define the site of interaction of CD46 with the MV envelope proteins, hybrid CD46/CD4 molecules containing SCR I, SCR I-II, or all four SCRs were generated. The SCRI-II-CD4 hybrid, but not the SCRI-CD4 hybrid, fulfilled MV receptor function.

S14-32

EXPRESSION OF THE EXTRACELLULAR PART OF THE CELL ADHESION MOLECULE E-CADHERIN/UVOMORULIN IN AN ARTIFICIAL PENTAMERIC FORM

Andrea Tomschy & Juergen Engel, Department of Biophysical Chemistry, Biocenter Basel

Formation of stable cell-cell contacts is always accompanied by cadherin clustering in cell contact sites. So far the adhesive function of the single extracellular fragment of E-cadherin could not be shown. To study the effect of clustering on the homophilic interaction a construct containing five extracellular fragments of E-cadherin was produced. Therefore the N-terminal part of rat cartilage oligomeric protein (COMP) was fused to the mouse cadherin fragment on the DNA level. The relevant DNA regions were amplified by PCR using the corresponding c-DNA clones as templates and inserted into the eucaryotic expression vector pRC/CMV. For expression human embryonal kidney cells were transfected. The recombinant protein is correctly expressed and is secreted only in its soluble pentameric form. Spectroscopic investigations reveal that the protein construct has native like properties. Its functionality will be tested in cell adhesion assays. Further biophysical characterization shall clarify how clustering of E-cadherin mediates homophilic interaction.

S14-33

METABOLISM OF CYCLOSPORIN A IN HUMAN AND RAT LUNG SLICES

M.C. Spaans, R. Fisher, K. Brendel, and A. Vickers. *Drug Safety, Sandoz Pharma Ltd., Basle Switzerland. Dept. Pharmac. and Tox., Univ. of Arizona.*

This study demonstrates the use of human and rat lung slices in comparison to liver for gaining insight of lung biotransformation of cyclosporin A (CSA), a promising asthmatic drug. Human lung slices (~ 400-500 μm ϕ), rat lung and liver slices (~ 200 μm ϕ) were prepared from 8 mm diameter cores using a Krumdieck Tissue slicer. The lungs were inflated with agarose (0.8-1%) which is soluble at 25-30 °C. After a preincubation of 1.5 hr, medium containing ^{3}H-CSA (3 μCi/ml, 1 and 10 μM) was added for 24 hr. Lung slices from 1 human metabolized CSA to the primary hydroxylated metabolite AM1, whereas rat lung (n=2) and liver (n=2) slices metabolized CSA to the primary hydroxylated metabolites AM1, AM9, AM4N, and the secondary metabolite AM19. The extent of total metabolism in human lung slice cultures for 24 hr was 47 pmol/mg slice protein for 1 μM CSA and increased 5-fold for 10 μM CSA, whereas in rat lung slices the extent was 306 pmol/mg slice protein for 1 μM CSA and increased 12-fold for 10 μM CSA. Based on these initial data the order of extent of CSA metabolism was rat lung ≥ rat liver ≅ human liver > human lung for both 1 and 10 μM CSA.

S14-34

STUDY OF THE PROTEIN INTERACTION OF P37K, THE MAJOR ENVELOPE PROTEIN OF VACCINIA VIRUS.

Schmutz, C., and Wittek, R.
Institut de Biologie Animale, Université de Lausanne, CH-1015 Lausanne

All vaccinia virus envelope proteins, apart from the hemagglutinin, are required for the formation of extracellular enveloped viruses (EEV). Furthermore, the 14K protein, present on the surface of the intracellular mature viruses (IMV) is also required for envelopment. P37K is the most abundant protein of the vaccinia virus envelope. Its biochemical characteristics and location suggest a similar functional role as the one played by the matrix protein present in several enveloped viruses.
Little is known about the protein interactions involved in the process of envelopment. Therefore, immunoprecipitations and Western blot analysis were performed on infected cell lysates as well as on EEV to study potential protein interactions of P37K. A high molecular weight protein complex of about 80 kD linked by disulfide bridges was precipitated from EEV particles but not from infected cell lysates. Western blot analysis revealed that the 42K envelope protein was not present in this complex. The isolation and dissociation of the 80 kD protein complex strongly suggested that it is composed of a P37K dimer.

S14-35

IN VITRO RECONSTITUTION OF RECOMBINANT SECRETORY IMMUNOGLOBULIN A

Blaise Corthésy and Lorenz Rindisbacher
Institut de Biologie animale de l'Université de Lausanne

Secretory IgA (sIgA) is the major class of antibody produced by mucosal tissues lining the digestive, respiratory and urogenital tracts. It consists of two IgA monomers and one joining (J) chain synthesized in plasma cells, plus one molecule of secretory component (SC) derived from the epithelial cells of mucosal surfaces. *In vitro*, IgA dimers are recovered and purified from secreting hybridoma, while SC is produced using virus-based expression systems developped in the laboratory. In this communication, we show that purified IgA and SC polypeptides from human and murine origin reassociate spontaneously under the form of sIgA complexes. Furthermore, we demonstrate that the partnership occuring between SC and IgA increases stability toward protease degradation when compared to IgA alone. Together, our data establish *in vitro* reconstituted sIgA as an appropriate source to asign a physiological role to SC both *in vitro* and *in vivo*.

Quality assessment of animals in experiments

S15-01

Immunosuppression by virus infection: Models of pathogenesis and dangers for experiments

ROLF M. ZINKERNAGEL
Institute of Experimental Immunology, University Hospital, 8091 Zurich

Analysis of the influence of virus infections on immune responses in mice to a third party antigen or the virus itself reveal that cytopathic viruses (e.g. vaccinia virus) may cause immunosuppression directly by destroying lymphohemopoietic cells involved in immune responses and/or by destroying the architecture of lymphoid organs. Non-cytopathic viruses (e.g. lymphocytic choriomeningitis viurs, LCMV) may cause a severe immunosuppression via immunopathological CD8+ T cell mediated destruction of antigen presenting cells and of the lymphoid structures. The latter mechanisms seems to be at least in part responsible for the long delay in neutralising antibody responses against LCMV and possibly may explain some aspects of HIV induced AIDS. Obviously, such induced modulations of immune responses, if unrecognized, may render experiments uninterpretable.

S15-02

CONTAMINATION OF TRANSPLANTABLE TUMOURS BY MURINE VIRUSES

Nicklas, W., Central Animal Laboratories, German Cancer Research Centre, Heidelberg, FRG

Transplantable tumours were tested for murine viral contamination by using the mouse/rat antibody production test. Of more than 350 tumours examined, about 20% were contaminated. Contamination with rodent viruses was most frequently detected in tumours of mouse origin; hamster or rat tumours were less frequently contaminated. Murine viruses were, too, found in human tumours after mouse passages. Considerable differences in the contamination rate became evident when transplantable tumours from in vitro and from in vivo passages were compared. The most frequently detected contaminant was lactic dehydrogenase elevating virus (LDV) which causes a lifelong lasting viremia in infected mice. In addition, murine parvoviruses (MVM, KRV), mouse hepatitis virus (MHV), reovirus 3, and the zoonotic lymphocytic choriomeningitis (LCMV) virus were found.

S15-03

IMMUNOLOGICAL AND MOLECULAR CHARACTERIZATION OF MICROSPORIDIA FROM HUMANS AND ANIMALS

Mathis A., Tanner I., Weber R., and Deplazes P.
Institute of Parasitology, University of Zürich.

Microsporidia are protozoan parasites with a broad host range including invertebrates and vertebrates. Emerging significance as opportunistic parasites in AIDS patients was reported for 3 species: *Encephalitozoon hellem, Septata intestinalis, Enterocytozoon bieneusi*. Seven microsporidian isolates were obtained from AIDS patients. *E. cuniculi* was isolated from nine rabbits. All isolates of rabbits and 5 of 7 from humans could be cultivated in MRC-5 cells. All isolates were characterized by Western blot (WB) and by restriction enzyme analysis (REA) of the PCR amplified SSU rRNA gene. All rabbit *E. cuniculi* isolates showed homologous (WB) and identical (REA) banding patterns. The analysis of three isolates from humans showed congruence with an *E. hellem* reference isolate (CDC:0291:V213). The banding patterns of an *Encephalitozoon*-like and of an *Enterocytozoon* isolate (isolated from stool) were unique among all tested isolates. The patterns of two isolates from AIDS patients were indistinguishable from the rabbit *E. cuniculi* isolates. This is a confirmation that *E. cuniculi* is a zoonotic parasite.

S15-04

EDUCATION AS A PREREQUISITE FOR ANIMAL WELFARE AND QUALITY OF EXPERIMENTS

L.F.M. van Zutphen, Dept. of Laboratory Animal Science, Utrecht, NL

During the last decade many contries have adopted legislative regulations on animal experimentation. All these laws are based on the premise that for certain purposes and under restricted conditions the use of animals for reseearch and testing is morally acceptable. One of the restrictions deals with requirements on competence of persons involved in animal experiments. It is generally recognizeed now that education and training are major prerequisites for the proper and human use of animals. Priority should be given to programmes for the (young) scientist as the responsible personne for the design of experiments.
According to the Federation of European Laboratory Animal Science Associations (FELASA) scientists should not be allowed to take the responsability for an animal experiment unless such a person is graduated (at the level of MSc or equivalent) in one of the biomedical sciences and, in addition, has finished a course on laboratory animal experimentation and the search for alternatives.
At Utrecht University, the organization of training courses on laboratory animal science for "new" scientists had its start in 1986. Since then more than a hundred of these 3-week courses have been organized for in total more than 2500 students. Information will be presented on the contents and educational approach of these courses.

S15-05

SELECTED ENDO- AND ECTOPARASITES POTENTIALLY AFFECTING ANIMAL EXPERIMENTATION.

B. Gottstein, Institute of Parasitology, University of Berne.

Although we basically know about the potential spontaneous occurence of protozoan and metazoan organisms in laboratory animals, information respective to pathogenicity is often lacking and what is available is scattered. Especially the use of immunodefficient or immunosuppressed animals may change the pathogenetic pattern considerably. The major parasites that may affect inner organs include *Toxoplasma gondii*, *Encephalitozoon* sp., *Pneumocystis carinii* and metacestodes among others; for the gastro-intestinal tract, we expect predominantly flagellated or apicomplexan protozoa, oxyurids and some others helminths; ectoparasites: mainly mites, lice and fleas. Two recent outbreaks in Switzerland, one concerning *Hexamita muris* and another concerning *Myocoptes musculinus*, will be discussed. As most parasitic infections include a chronic clinical or subclinical course of disease, they may be difficult to diagnose but will affect biological parameters of the host animal considerably.

Medical biochemistry, physiology and toxicology

S16-01

F(ab')$_n$ prepared using precision conjugation techniques.

R. C. Werlen*, M. Lankinen, A. Smith, R. E. Offord and K. Rose.

It has recently become easily possible to prepare homogeneous artificial proteins by self-assembly of aminooxy peptides on a poly-aldehyde template through the formation of oxime bonds (1). We have transported this approach to the construction of F(ab')$_n$ conjugates of controlled structure. Using the method described in (2), we prepared and characterized by ESI-MS an Fab' fragment of an anti-CEA murine IgG1 monoclonal antibody having a single thiol group close to the C-terminus of the truncated heavy chain rather than the three normally obtained. We then introduced a unique amminooxy group into this Fab' by an appropriate substitution of the thiol group. The aminooxy Fab' molecules were then assembled into the F(ab')$_2$ or F(ab')$_3$ form by oxime formation with a di- or trialdehyde in two steps. The F(ab')$_3$ was found to have full immunoreactivity and an association constant 5 times higher than the Fab' and twice that of the F(ab')$_2$. By replacing one of the three aldehydes of the trialdehyde by a different reactive group, we have been able to make F(ab')$_2$ molecules with a single reactive group of our choice. For instance, we prepared in this way a mono-maleoyl F(ab')$_2$ with which we then reacted a further molecule of the Fab' mono thiol, leading to the formation of an F(ab')$_3$ with an alternative framework structure.

S16-02

IMMUNOFLUORESCENCE WITH MULTIPLE RABBIT ANTISERA

Landmann, L., Anatomisches Institut der Universität, 4056 Basel

Probing of more than one antigen in the same specimen depends on the availability of primary antibodies (AB) from different species or of different subclasses. Therefore, semithin cryosections of rat liver were probed sequentially with monospecific rabbit antisera. AB were visualized with fluorochrome-conjugated secondary AB or protein A (pA). In order to prevent crossreactivity AB and/or pA were inactivated by treatment with aldehydes between incubations. Fluorescence was recorded on a confocal laser scanning microscope and evaluated for staining intensity and crosstalk using the multicolor analysis software (Leica Lasertechnik). Labeling with fluorochrome-conjugated secondary AB resulted in considerable crosstalk. Denaturation of the first AB-pair with increasing concentrations of aldehydes (up to 8% paraformaldehyde/1% glutaraldehyde) decreased crosstalk but did not abolish it completely. Labeling with fluorescent pA resulted in crosstalk-free visualization of antigens if free pA was used as a blocking step between the incubations. However, as compared to indirect immunofluorescence, this method has the disadvantages of reduced staining intensity and decreased stability of the AB-pA complexes which can be improved by 0.1% glutaraldehyde but not by 4% paraformaldehyde. Conclusion: Two or more antisera can be visualized without crosstalk in the same specimen by pA conjugated to fluorochromes. Supported by SNF grant # 32-40486.94

S16-03

BIOCOMPUTING ON A SERVER NETWORK

R. Doelz, F. Eggenberger and Ch. Wadley, Biozentrum der Universität, CH-4056 Basel

We have observed an increasing discrepancy between the computer resources available locally and those accessible via networks. As these are developing in a divergent rather than an integrated fashion, the researcher faces three different worlds and environments which are mutually exclusive: local (PC/Mac), on-site (Computing Center) and world-wide networks. This phenomenon is typically causing confusion within electronic mail server environments, and the recent developments on the World-Wide Web (WWW). We have, therefore, developed the Hierarchical Access System for Sequence Libraries in Europe (HASSLE) [1,2] which enables any computer running the necessary client program to connect to one of the nodes which run a HASSLE server. Using HASSLE software allows molecular biologists to work within their well-known environment without change, as the networking is layered below the user interface part of the program. The most important aspect of automatic resource discovery is based on the concept of fault-tolerant, redundant services. This principal is novel and improves the established concepts of the currently used mechanisms greatly. The sophisticated modus of HASSLE operation allowed us to break the major weakness of the currently available client/server technologies: HASSLE is neither synchronous or bound to a peer-to-peer communication model, which implies that the answer to a given question may even come from a different host than the originally approached server. HASSLE implements both access control and security. All HASSLE communication is encrypted. HASSLE is now used regularly at many European Molecular Biology Network (EMBnet) nodes. It has proven to be a superior method for access to a network of servers in an integrated environment. The software is freely available. The project was funded by Basel University and grants from the Schweizerischer Nationalfonds, the Bundesamt für Bildung und Wissenschaft, and the European Union.

[1] Doelz, R. Computer Applications in the Biosciences (CABIOS) 10, 31-34 (1994).
[2] Doelz, R. Computer Networks and ISDN Systems 26 (Suppl. 4), 157-162 (1994).

S16-04

CHARACTERIZATION OF THE GENE FOR HUMAN NEUTROPHIL-ACTIVATING PEPTIDE 78 (ENA-78)

Max S. Corbett, Ina Schmitt*, Olaf Riess*, and Alfred Walz. Theodor Kocher Institute, University of Bern, CH-3000 Bern 9, Switzerland and the *Department of Molecular Human Genetics, Ruhr-University, Bochum, Germany

Epithelial-derived neutrophil-activating peptide 78 (ENA-78) was originally isolated and cloned in our laboratory from a IL-1β stimulated human pulmonary type-II-like epithelial cell line, A549. ENA-78 is a secreted protein of 78 amino acids and belongs to the C-X-C family of chemotactic cytokines. Members of this family include IL-8, NAP-2 and GRO. The genomic DNA for ENA-78 has been obtained from a human chromosome 4 flow-sorted cosmid library. Three out of 25'000 screened single colonies yielded the same 2.2 kB EcoRI ENA-78 gene fragment. A similar size fragment was observed on genomic southern blots suggesting the presence of a single ENA-78 gene. The transcriptional start site was localized by using a 5' RACE protocol on first strand cDNA prepared from stimulated alveolar type-II epithelial cell (A549) poly(A) mRNA. The ENA-78 gene contains four exons and three introns and the open reading frame of 342 nucleotides encodes for a protein of total 114 amino acids. The 5' flanking region contains potential binding sites for several nuclear factors such as AP-2, NF-κB, and interferon regulatory factor -1.

S16-05

Exopeptidase expression in brain microvessels is regulated by glial-derived factors

L Juillerat-Jeanneret, MF Hamou, F Monnet-Tschudi, MG Zurich, JI Murata,P Darekar, P Honegger. University of Lausanne. 1011 Lausanne

Tumor cells are dependent on angiogenesis for their growth. While the role of endoproteases, including matrix metalloproteases, in digesting extracellular matrix during the process of angiogenesis has received some attention, the role of exopeptidases has not been evaluated. We have measured the *in situ* enzymatic activities of amino(di)peptidases in vessels invading brain tumors in human histological samples and found that these vessels expressed high levels of an aminopeptidase which preferentially hydrolyzes substrates with an acidic N-terminal amino acid. In order to study the role of tumor-derived factors in regulating the expression of this enzyme, we have developed a tridimensional *in vitro* model consisting of rat brain cells in aggregate cultures which were supplemented with purified brain-derived microvessels. To these aggregates, we have added the medium conditioned by glial tumors in hypoxic culture conditions and analyzed the expression of aminopeptidases. Normal (no microvessels added) aggregates did not express aminopeptidase activity. Microvessels-enriched aggregates expressed aminopeptidase activity and this expression was further increased by the addition of hypoxic medium. These results suggest a role for hypoxic conditions in tumors in the regulation of proteolytic balance in the vascular system.

S16-06

THE GREEN FLUORESCENT PROTEIN: A NEW IN VIVO MARKER OF GENE EXPRESSION AND PROTEIN LOCALIZATION.

S. R. Kain, Cell Biology Group, CLONTECH Laboratories, Inc., Palo Alto, CA, USA 94303

The green fluorescent protein (GFP) from the jellyfish *Aequorea victoria* is rapidly becoming an important reporter molecule for monitoring gene expression and protein localization *in vivo* and in real time. GFP emits bright green light (λmax = 509 nm) when excited with UV or blue light (λmax = 395 nm). The absorption and emission spectra of GFP are similar to those of fluorescein, and the conditions used to visualize this fluorophore are also suitable for GFP. Unlike other bioluminescent reporters, the chromophore in GFP is intrinsic to the primary structure of the protein, and GFP fluorescence is stable, species-independent, and can be monitored noninvasively in living cells by either fluorescence microscopy or FACS analysis. There are no apparent toxic effects of GFP expression in bacteria or eukaryotes. GFP fluorescence can also be detected in transparent whole organisms such as *C. elegans* and *Drosophila*. Here we demonstrate the fluorescence of GFP in various bacterial and eukaryotic cells and introduce a series of reporter vectors for GFP. These include a promoterless GFP vector for monitoring the expression of cloned promoters in mammalian cells, and a series of six vectors for creating fusion proteins to either the amino or carboxy terminus of GFP.

S16-07

N-linked glycosylation in yeast : the essential gene *SLW1* is required for the transfer of the core oligosaccharide to asparagine residues *in vivo*

R. Zufferey, G. Reiss, P. Burda, S. te Heesen, M. Aebi
Mikrobiologisches Institut ETH Zürich

The oligosaccharyltransferase complex (OTase) catalyzes the transfer of the core oligosaccharide from the lipid dolichol to selected asparagine residues of nascent poplypeptides. In a screen designed to detect proteins necessary for this process we have identified the SLW1 locus, encoding a putative membrane protein of 90 kDa. Depletion of this protein results in underglycosylation of secretory proteins and loss of OTase activity as measured in microsomal exctracts from depleted cells. Though not present in purified OTase complex (JBC 269 : 12908), SLW1 appears to be required for OTase activity *in vivo*.

S16-08

CHARACTERIZATION OF FLIP70, A FIBRINOGEN-LIKE PROTEIN SECRETED BY T CELLS.

C. Rüegg[1], S. Blum[1], D. Gundersen[1], M. Schreyer[2], V. von Fliedner[2], R. Pytela[3]. [1]CPO, CHUV, [2]Ludwig Institute, Lausanne, [3]UCSF, San Francisco, U.S.A.

We cloned a human cDNA coding a protein similar to fibrinogen β and γ chains and homologous to the previously identified murine gene *pT49*. By Northern blot analysis, flip70 mRNA is highly expressed in peripheral blood T cells. By RT-PCR on FACS-sorted cells flip70 mRNA can be amplified from the $CD4^+/CD8^-$ and $CD4^-/CD8^+$ T cell subsets. To identify the protein we generated monoclonal antibodies against a synthetic peptide based on the deduced C-terminal sequence. The antibody 23A6 precipitates a glycoprotein of 70 kDa MW (red SDS-PAGE) from the culture supernatant of peripheral blood resting T cells. Unreduced material migrates with a MW of over 400 kDa, suggesting that flip70 may be secreted as a multimeric complex. T cell activation results in a rapid down regulation of flip70 mRNA expression and protein secretion. Using mab 23A6 we studied flip70 expression in colon tissue. The majority of lamina propria T cells and their surrounding stroma are flip70 positive, while only a few of the lymphocytes in lymphoid tissues are positive.

S16-09

DEVELOPMENTALLY REGULATED EXPRESSION OF α2,8 LINKED POLYDEAMINONEURAMINIC ACID IN RAT KIDNEY: COMPARISON WITH α2,8 LINKED *N*-ACETYLNEURAMINIC ACID

Qu, B.*, Ziak, M.*, Zuber, C.*, Li, W*, Kanamori, A., Kitajima, K., Inoue, S., Inuoe, Y., and Roth, J.* *Division of Cell and Molecular Pathology, Department of Pathology, University of Zurich, Zurich, Switzerland, Department of Biophysics and Biochemistry, University of Tokyo, Tokyo, Japan

α2,8 linked polydeaminated neuraminic acid (α2,8 polyKDN) was discovered in rat kidney by immunohistochemistry and Western blotting using the mab kdn8kdn. In developing and adult rat kidney, α2,8 polyKDN was detectable in various stages of nephron development and all parts of the differentiated urinary tubule albeit at different intensity. Specificity of labeling was demonstrated by pretreatment with KDNase. A single reactive 300 kD band was detectable in kidney. The developmental expression profile and cellular distribution pattern of α2,8 polyKDN and of α2,8 polysialic acid of NCAM differed greatly.

S16-10

OCCURRENCE OF α 2,8 POLYDEAMINONEURAMINIC ACID IN MAMMALIAN TISSUES: WIDESPREAD AND DEVELOPMENTALLY-REGULATED BUT HIGHLY SELECTIVE EXPRESSION ON GLYCOPROTEINS

Ziak, M*, Qu, B*, Zuo, X*, Zuber, C*, Kanamori, A., Kitajima, K., Inoue, S., Inuoe, Y., and Roth, J.* *Division of Cell and Molecular Pathology, Department of Pathology, University of Zurich, Zurich, Switzerland, Department of Biophysics and Biochemistry, University of Tokyo, Tokyo, Japan

A unique deaminated *N*-acetylneuraminic acid, KDN (2-keto-3-deoxy-D-*glycero*-D-*galacto*-nonulo-sonic acid), was recently discovered in rainbow trout egg polysialoglycoprotein. We report the presence of α2,8 polyKDN-bearing glycoproteins in various mammalian tissues with the use of the mouse monoclonal antibody mAb kdn8kdn and a new bacterial sialidase, KDNase SM. The α2,8 polyKDN glycan was found in almost all studied tissues and in some showed a developmentally regulated expression. Surprisingly, by Western blotting only a single band was detectable in given tissue.

S16-11

CONSTRUCTION OF A SINGLE-CHAIN F_V-FRAGMENT OF THE CHIMERIC ANTI-NEUROBLASTOMA ANTIBODY CHCE7

H. Amstutz, F. Carrel*, J.-J. Morgenthaler, Central Laboratory, Swiss Red Cross, 3000 Bern 22, *Paul Scherrer Institute, 5232 Villigen-PSI.

The monoclonal antibody CE7 shows strong tumor selectivity by binding to a neuroblastoma-associated cell surface antigen. In order to develop a single-chain F_V fragment with the CE7 specificity, an expression cassette containing the pelB leader sequence and a hexa histidine tag for easy purification was assembled by PCR. The CE7 V_H and V_L genes were expressed in the cassette and growth conditions for the secretion of the scF_V were optimized. In JM101 there was no scF_V production and viability of the cells was very low. The cotransfection of the expression vecor pC2 with the plasmid pRep4 containing the lac i gene strongly improved the viability of the cells. E. coli strains Topp1-6 (Stratagene) were transfected with pC2 and pRep4 and screened for scF_V expression. Variable amounts of expression levels were seen under the usual growth conditions. No secreted scF_V was detectable. At lower growth temperatures, secreted scF_V started to appear in the culture supernatant with an optimum at 22°C. Ni-NTA-Agarose was used for the affinity purification of the protein with a yield of 3 mg of purified scF_V protein per liter. Preliminary results showed binding to neuroblastoma cell lines with a K_D of $6x10^{-9}$ M at 37°C.

S16-12

PATTERNS OF LOCAL ANTIBODY RESPONSES FOLLOWING MUCOSAL IMMUNIZATION OF MICE VIA DIFFERENT ROUTES WITH A *SALMONELLA* RECOMBINANT VACCINE EXPRESSING HEPATITIS B VIRUS NUCLEOCAPSID.

S. A. Hopkins[1], J.- P. Krähenbühl[1], F. Schödel[2], A. Potts[3], P. De Grandi[3] and D. Nardelli-Haefliger[3]
Inst. of Biochemistry, UNIL[1]; Dpt. of Gynecology, CHUV[3], CH-Lausanne and INSERM U 80, Hôpital Edouard Herriot, F-Lyon[2]

Mouse immunization with a *Salmonella* attenuated strain $PhoP^c$ expressing the Hepatitis B virus nucleocapsid (HBc) induces specific antibody responses against bacterial LPS and HBc. Different routes of immunization have been compared for their ability to induce systemic and mucosal responses at different sites. Anti-LPS and Anti-HBc IgA antibodies have been measured in saliva, genital and intestinal secretions and feces. All routes were able to induce to variable extents specific antibodies in serum and secretions. The vaginally immunized mice fell into two groups, a high responding and a low or non responding group which correlated with the estrous cycle at the time of immunization. In serum, saliva and genital secretions the highest anti-HBc titers were reached using the nasal route followed by the oral route, while the highest anti-LPS titers were obtained by the vaginal route. The rectal route induces the highest titers in colo-rectal secretions and feces. Our data indicate that using live attenuated *Salmonella* it is possible to design vaccination strategies aimed to elicit secretory antibody responses at specific locations.

S16-13

DIVERSE TYPES OF MUCINS RECOGNIZED BY L-SELECTIN CARRY SIALYLATED, SULFATED POLYLACTOSAMINES, BUT HIGH AFFINITY BINDING CANNOT BE EXPLAINED BY LINEAR OLIGOSACCHARIDE SEQUENCES

P.Crottet, Y.J.Kim, and A.Varki
UCSD Cancer Center, La Jolla, CA 92093

Previous studies have shown that the polypeptides CD34, GlyCAM-1 and MadCAM-1 can function as ligands for L-selectin (LS) only when synthesized by the specialized high-endothelial venules in lymph nodes. Since sialylation, sulfation, and possibly fucosylation are required for generating recognition, we reasoned that other mucins known to have such components might also act as ligands. We found that mucins secreted by human carcinoma cells and bronchial mucus can bind to LS in a calcium-dependent manner. A comparative study with GlyCAM-1 indicated that in all three cases: 1) α2-3 linked sialic acids are required for binding; 2) only a subset of the molecules carries functional ligands; 3) binding persists after protease fragmentation and boiling, indicating the primary role of carbohydrates in recognition; 4) despite this, O-linked glycans released from the ligands do not show detectable binding to LS. Together with studies using an array of endoglycosidases these data suggest that mucins with sialylated, sulfated lactosamine-type O-linked chains can generate high-affinity LS ligands, but only when they present these chains in unique combinations presumably dictated by the polypeptide backbone. Given the small size of the LS carbohydrate-binding domain relative to potential oligosaccharide ligands, high affinity recognition is likely to result not from multivalency but from the generation of a limited number of "clustered saccharide patches".

S16-14

RATES OF PHAGOCYTOSIS OF ORTHOROMBIC CYCLOSPORINE A (CsA) AND LATEX PARTICLES OF ALVEOLAR MACROPHAGES FROM HAMSTERS

I. Maye[1], A. de Fraissinette[2], P. Gehr[1], J. Vonderscher[2] and F. Richter[2]. [1] Institut of Anatomy, University of Berne, Berne and [2] Sandoz Pharma AG, TRD Drug Delivery Systems, Basel, Switzerland.

We investigated the rates of phagocytosis of orthorombic Cyclosporine A (CsA) particles and latex particles in alveolar macrophages from hamsters. The macrophages were collected by broncho-alveolar lavage (BAL) and incubated with 2 different doses of both particles (0.1mg and 0.5mg) at 3 incubation times (1h, 6h, 24h). Macrophages without particles were cultured in parallel. The cultures were embedded in Epon and further processed for light and transmission electron microscopy. A morphometric method was established to assess the volume of phagocytized particles per volume of macrophages. Phagocytosis of CsA was dose and time dependent only if the incubation time was > 6h. At 24h, the macrophages incubated with 0.5mg CsA had ingested 2 times more particles than at 1h and 4 times more than those macrophages incubated with 0.1mg. The rate of phagocytosis of latex particles was higher than that of CsA particles and in a dose and time dependent manner. For all incubation times, the rate of phagocytosis of macrophages incubated with latex particles at 0.5mg was higher than that of those incubated at 0.1mg. At 24h, the macrophages incubated at higher dose had ingested 3 times more latex particles than at 1h. These differences observed between CsA and latex particles will be further discussed in order to better understand the process of phagocytosis of BAL macrophages.

S16-15

EFFECT OF INORGANIC SALTS ON GLYCOGEN SYNTHESIS IN MUSCLE STRIPS PREPARED FROM RAT DIAPHRAGM

Silvia Maggini, Hanni Zürcher and Paul Walter, Department of Biochemistry, University of Basel, Vesalianum, Vesalgasse 1, CH-4051 Basel, Switzerland

Previous studies carried out in this laboratory have shown that glycogen deposition in liver and muscle is significantly greater in rats fed a diet containing barley malt extract than in those fed glucose, starch or the carbohydrate components of the malt extract. The malt extract (glucose oligomers, inorganic salts, protein) was shown to have several dietary advantages on glucose absorption and deposition. One effect was a greater glycogen deposition in muscle due to the presence of inorganic salts in the malt extract (Flückiger-Isler *et al.*, (1994) J. Nutr. **124,** 1647-1653). In order to determine the mode of action of the inorganic salt mixture on skeletal muscle, experiments with isolated muscle tissue were performed. Muscle strips prepared from rat diaphragm were incubated in Trowell's T8 medium. Less than 10% of total muscle creatine kinase activity was recovered in the incubation medium during the whole incubation period of 6 hours, showing a good integrity of the preparation. During the same incubation period, nucleotide levels were shown to remain stable and the rate of glycogen deposition to increase linearly with time. The effects of individual components of the salt mixture as well as of the whole mixture on the rate of glycogen synthesis were investigated. The results indicate that both magnesium and calcium supplementation of the incubation medium resulted in a significant increase of glycogen deposition ranging between 15% (Mg^{2+}) and 50% (Ca^{2+}).

S16-16

INDUCTION OF UNIDIRECTIONAL BLOCK AT ABRUPT EXPANSIONS OF EXCITABLE TISSUE BY LOCAL SUPERFUSION WITH TTX OR VERAPAMIL: MULTISITE OPTICAL RECORDING IN PATTERNED GROWTH MYOCYTE CULTURES

Rohr S., Kucera J.P., Dept. of Physiology, University of Bern, Bern, Switzerland

In order to dissect the ionic currents involved in successful propagation of an action potential across an abrupt tissue expansion, we constructed expanding cellular structures in vitro using patterned monolayer cultures of neonatal rat heart cells and followed impulse propagation across the expansion using multiple site optical recording of transmembrane voltage (MSORTV). The preparations, which consisted of narrow cell strands (20-80 µm wide) inserting into large rectangular cell areas (side lengths >2 mm), were stained with the voltage-sensitive dye di-8-ANEPPS and were imaged onto a linear array of 22 photodetectors (spatial resolution of 25 µm in the object plane using a 40x objective). The preparations were stimulated by extracellular electrodes from either side of the expansion and a local superfusion (300 to 500 µm wide) containing either 10 µM TTX or 10 µM verapamil was juxtaposed to the expansion over the narrow cell strand. As shown previously, impulse propagation in anterograde direction (narrow -> wide region) across expansions under control conditions was characterized by a local activation delay ($d_{control}$) in the range of 0.5 to 2 ms. Suppression of the sodium inward current with TTX proximal to the expansion invariably induced conduction block in anterograde direction and delayed excitation along the superfused segment in retrograde direction (n = 4). If $d_{control}$ was in the range of 1 to 2 ms, a unidirectional block in anterograde direction was also elicited by superfusing the narrow cell strand with verapamil (n = 3). If $d_{control}$ was shorter, verapamil induced a prolongation thereof without induction of block. Our results show that both sodium and calcium inward currents affect the safety of propagation across an abrupt expansion of excitable tissue.

S16-17

Optical Mapping of Impulse Conduction in Anisotropic Neonatal Rat Heart Cell Monolayers

Vladimir G. Fast, André G. Kléber, University of Bern, Bern, Switzerland

Anisotropy of cardiac muscle plays an important role in the propagation of excitation and in the generation of reentrant arrhythmias. Maximal rate of depolarization (dV/dt_{max}), safety factor, and susceptibility to conduction block have been shown to depend on the direction of impulse conduction. To study anisotropic activation spread at a microscopic level, we have previously developed a method for culturing anisotropic rat heart cell monolayers on a growth-directing substrate of collagen. Here, we used an optical mapping system with a 10x10 photodiode array and a voltage-sensitive dye (RH-237) to assess anisotropic activation spread, conduction velocity, and distribution of dV/dt_{max} with a spatial resolution of 6 to 15 µm. In dense monolayers, the spatial inhomogeneity of activation maps was more pronounced during longitudinal (L) than during transverse (T) conduction which was related to the anisotropic architecture. In average, L conduction velocity was two times larger than T velocity. In contrast, no directional differences in average dV/dt_{max} were detected. In monolayers with low cell density, the presence of longitudinally oriented clefts between cells resulted in major discontinuities of conduction. Transverse dV/dt_{max} increased before and decreased beyond the clefts with respect to its value during L spread. We conclude that the anisotropic cell structure results in the directional differences in microscopic inhomogeneity of activation spread and differences in conduction velocity. By contrast, anisotropy does not result in directional differences of dV/dt_{max}. They occur only in presence of major discontinuities in tissue architecture.

S16-18

Role of Tumour Necrosis Factor-α in altered energy balance in response to lipopolysaccharide administration : evidence from Transgenic *soluble TNF-R1* mice.

Denis Arsenijevic*, Irene Garcia**, Richard Benzi*, Josiane Seydoux*, Lucien Girardier* and Abdul G. Dulloo*
Department of Physiology, Department of Pathology***
Centre Médical Universitaire, 1211 Geneva 4, Switzerland*

The importance of TNF-α in LPS-induced alterations in energy balance during acute-phase reactions was investigated by comparing the food intake and energy expenditure (assessed by indirect calorimetry) of transgenic mice expressing soluble TNF-R1 and their negative littermates.

The results indicate that in response to LPS injection (250µg), the transgenic mice lost less body weight, had shorter duration of anorexia, and showed a reduced thermogenic response compared to control mice.

These findings provide direct evidence that TNF-α plays a role in the anorectic and thermogenic mechanisms underlying LPS-induced weight loss. The fact that these responses were only *partially* abolished in the transgenic mice suggests that, in addition to TNF-α, other factors (e.g. other cytokines) are also implicated in LPS-induced catabolic effects.

S16-19

WHICH NON-INVASIVE METHOD MONITORS BETTER GLUCOSE-INDUCED SYMPATHETIC ACTIVATION IN HUMANS?

Farinelli C.C.J., Dulloo A.G. and Girardier L.
Department of Physiology, CMU, CH-1211 Geneva 4, Switzerland

This study examined two non-invasive indices of cardiac sympathetic activity in response to a bolus of 75 g of glucose in humans: (1) spectral analysis of heart rate variability (HRV) which provides both a pure marker of vagal tone and an index of changes in sympatho-vagal balance, (2) the ECG's T-wave amplitude (TWA), which is decreased by β-adrenergic stimulation of the heart. While remaining in the supine position, ECG and respiratory movements were recorded in 8 healthy male subjects before and during 2 hours after the ingestion of glucose, and their metabolic rates were measured by indirect calorimetry. The experiment was performed in duplicate on two separate days in each subject. The results indicate that the increase in metabolic rate induced by glucose ingestion is concomitant with a decrease in TWA in all subjects on both occasions. In contrast, individual changes in the two indices obtained by spectral analysis of HRV were much more variable, with only two subjects showing an increase in sympathetic activity on duplicate measurements. These results indicate that the change in TWA is a more reliable marker to monitor glucose-induced sympathetic activation.

S16-20

Characterization of myomesin as an essential protein in myofibrils

Bantle, St.; Perriard, E., and Perriard J.-C.,
Institute for Cell Biology, ETH, 8093 Zürich.

Myomesin is a 185 kD myofibrillar protein which is located in the middle of the sarcomeres, and is one out of the three known M-band proteins: M-protein, myomesin and the muscle specific isoform of creatine kinase. It has been speculated that myomesin might bridge the gap between the titin filament system and the thick-filament system at the M-band, and therefore, it seems to be essential for the fundamental architecture of myofibrils. The aim of this project is to identify cDNA clones of myomesin which will lead to the protein sequence and enable us to examine functional aspects.

A chicken heart muscle λ-gt 11 cDNA expression library was screened by DNA hybridization and by a monoclonal antibody against myomesin. We have identified a cDNA of over 6000 bp including one open reading frame of ~4200 bp. Sequence comparisons between our heart specific cDNA and a chicken skeletal muscle cDNA fragment suggest a heart specific C-terminus for myomesin. This data confirms the earlier observations of two different patterns on immunoblots of tissue extracts as well as northern blots. According to the results on southern blots we have now the hypothesis of two isoforms, being the product of one gene. We suggest an alternative splicing. (Supported by SNSF grant 31-37537.93)

S16-21

Chronic Oral Musk Xylene Specifically Induces Cytochrome P4501A in Long Evans Rats

R.Suter, U.Boelsterli[1], W.Lichtensteiger, M.Schlumpf. *Inst. of Pharmacology, University of Zürich, Zürich; [1]Inst. of Toxicology, ETH and University of Zürich, Schwerzenbach*

The synthetic fragrance musk xylene, a lipophilic and highly resistant compound, is widely used in perfumes, detergents, soaps and lotions. It bioaccumulates in fish and has been detected recently also in human fat and milk. Fat concentrations and liver enzyme induction (Cyp 1A2, Cyp 1A1, O-dealkylation of methoxy- and ethoxyresorufin (MROD, EROD)) were analyzed in Long Evans rats fed chronically for 7 weeks with food pellets containing 0,1g/kg of the pollutant. MROD and EROD were determined in microsomal preparations of liver homogenates by spectrofluorimetry and musk xylene concentrations in fat was analyzed by gas chromatographie with ECD-detection. EROD activity was increased 2 to 3 fold in animals fed on 0,1g/kg musk xylene. EROD activity was also increased by about the same extend in 14 day old pubs, whose mothers have been fed on 0,1g/kg musk xylene pellets for 6-7 weeks before mating, during pregnancy and after birth.

S16-22

VITAMIN E AFFECTS PHARMACOKINETICS AND EFFECTIVENESS OF DESIPRAMINE AND OTHER CATIONIC-AMPHIPHILIC DRUGS IN VITRO

Ulrich E. Honegger, Isabel Scuntaro, Department of Pharmacology, University of Bern, CH-3010 Bern

Chronic exposure of confluent cell cultures (human fibroblasts, rat astrocytoma cells) to therapeutic concentrations of the tricyclic antidepressant desipramine (DMI) led to a strong drug accumulation in the lysosomal cell compartment. This cationic-amphiphilic compound inhibited cellular phospholipid metabolism and thus induced quantitative and qualitative changes in the cellular and membraneous phospholipid compositions. It fluidized plasmamembranes and reduced β-adrenoceptor density. Co-administration of vitamin E (=D-α-tocopherol) diminished cellular uptake of DMI and its effects including β-adrenoceptor down-regulation. These in vitro findings might be suggestive for an influence of vitamin E on effectiveness of DMI and other cationic-amphiphilic drugs in vivo.
Supported by the Swiss National Science Foundation.

S16-23

VOLUMETRIC ANALYSIS OF HEPATOCYTES FROM RATS EXPOSED TO POLYCHLORINATED BIPHENYL CONGENER NUMBER 77

Gilroy, C., Peng, J., Suidgeest, P., Singh, A. and Ireland, W. Atlantic Veterinary College, Charlottetown, PE, C1A 4P3, Canada.

Sprague-Dawley rats (9 males & 9 females) were fed PCB congener 77 for 91 days, and were used in the study of liver toxicity by quantitative electron microscopy. Resin-embedded sections from livers of three males or females of control, 10 (lowest) and 10,000 (highest) ppb groups that contained a terminal hepatic venule were selected. Image analysis was performed on a "Bioquant IV" digital morphometry system. Optical microscope images of the fields were projected on a monitor by a video camera. Hepatocytes of zone 3 were sampled randomly with a point grid. Intercept lengths of each sampled cell were measured. From means of the intercepts cubed, a volume weighted mean volume of the hepatocytes was estimated by the method of Cruz-Orive (J. Microsc., *120*:15, 1980). Mean cell volume of control, 10 and 10,000 group males was calculated to be 5,676 ± 304 (S.E.M.), 6,439 ± 340 and 5,333 ± 267 μm^3, respectively; values for the corresponding females were 4,999 ± 264, 4,553 ± 252, and 4,865 ± 239 μm^3, respectively. Conclusion: hepatocytes in the treated male rats had significant difference (p = 0.032) in the cell volume between low and high dose exposures, but a significant difference (p = 0.437) was not detected in this value in the females.

S16-24

ETHINYLESTRADIOL INDUCES DIFFERENTIAL EFFECTS ON VARIOUS TRANSPORTER mRNA AND PROTEIN LEVELS IN RAT LIVER

H. Kupferschmidt, B. Hagenbuch, B. Stieger, St. Krähenbühl and P.J. Meier. Clinical Pharmacology and Toxicology, University Hospital, 8091 Zürich

Studies in liver plasma membrane vesicles have suggested that ethinylestratiol (EE) treatment of male rats induces distinct transport alterations in hepatocytes. Since several of the affected transporters have been cloned, we studied the effects of EE-treatment on the mRNA and partly on the protein levels of three basolateral (NaK-ATPase, Ntcp and oatp) and one canalicular (sat-1) transport systems. Pairfed male rats were treated with 1,2 propanediol (controls) or with 5 mg/kg EE (cholestasis) for 5 days. Rat liver mRNA was quantitated on Northern blots and microsomes were used for immunoquantitation of Ntcp and NaK-ATPase. In cholestatic livers Ntcp mRNA was decreased by 60%, whereas oatp, sat-1 and NaK-ATPase mRNAs stayed unchanged. mRNA of β-actin and GAPDH increased by 1.5-2 fold. On the protein level Ntcp decreased by 85%, whereas the α_1-subunit of NaK-ATPase decreased only slightly (~25%) in EE-treated as compared to control animals. These studies demonstrate that the well established decrease of hepatic Na^+-dependent bile acid uptake in EE-treated rats is a consequence of a reduced expression of Ntcp while mRNA levels of other proteins remain unchanged.

S16-25

CYANOBACTERIA TOXICOSIS: THE ZÜRICH CONNECTION

A. Sahin[1], F. G. Tencalla[2], D. R. Dietrich[2], K. Mez[3], K. Hanselmann[3], and H. Naegeli[1], [1]Institute of Pharmacology and Toxicology, [2]Institute of Toxicology, [3]Institute of Plant Biology, University of Zürich and Federal Institute of Technology

Hepatotoxic polypeptides produced by cyanobacteria (blue-green algae) cause poisonings of wild and domestic animals in many parts of the world. In addition, contamination of water supplies may lead to potential hazards to human health. Our three Institutes are engaged in a collaborative effort to facilitate diagnosis of animal poisonings by hepatotoxic cyanobacteria. We have developed a simple and fast method to extract cyanobacteria hepatotoxins from biological samples, including liver tissue obtained from affected animals. To detect these hepatotoxic metabolites we exploit their ability to selectively inhibit protein phosphatases from eukaryotic sources. This method has a detection limit for microcystin of ~1 ppb. (Supported by BUWAL and NF grant 31-33344.92 to D.R.D., by NFP31 40-33432.92 to K.H., and by grant 012.91.11 from The Bundesamt für Veterinärwesen to H.N.)

S16-26

EFFECTS OF THE HEPATOMITOGEN CYPROTERONE ACETATE ON NEGATIVE GROWTH CONTROL BY TGF-ß IN CULTURED RAT HEPATOCYTES

R.Fasciati, K.Frei, D.Dietrich and P.Maier, Institute of Toxicology, ETH and University of Zürich, CH-8603 Schwerzenbach

Exposure of rodents to Cyproterone Acetate (CPA) causes hepatocyte proliferation within 24 - 48 h and liver tumors after long term treatment. This suggests that negative growth control might be affected by the hepatomitogen CPA, which acts mainly periportally. Therefore the growth inhibiting activity and expression of TGF-ß1 and -2 were investigated in rat hepatocytes cultured at periportal-like conditions. Following a 48 h exposure to CPA, a dose dependent increase in DNA synthesis (^{3}H-TdR) and PCNA labeling index was observed. The maximum effects were attained with concentrations of 12 and 25 µM CPA, respectively. While a threshold level at 0.1 ng/ml TGF-ß1 and -2 for inhibition of CPA induced PCNA expression could be observed, ^{3}H-TdR incorporation was reduced over the whole dose range tested (0.01-1.0 ng/ml). In addition TGF-ß mRNA expression was investigated by RT-PCR analysis. TGF-ß2 and -3 are expressed by the cultured hepatocytes. The time dependent expression and the induction by CPA was investigated and compared with the activity of EGF as an endogenous growth factor. So far no qualitative differences between CPA and EGF induced expression of TGF-ß were found.

S16-27

THE HUMAN GENE CODING FOR THE E2 SUBUNIT OF THE PYRUVATE DEHYDROGENASE COMPLEX (E2-PDC), AN AUTOANTIGEN IN HALOTHANE HEPATITIS

Valeria Koch and Josef Gut, Department of Pharmacology, Biocenter of the University, CH-4056 Basel, Switzerland.

The anesthetic agent halothane is oxidatively metabolized by cytochrome P4502E1 to the intermediate CF_3COCl which covalently reacts with proteins, leading to the formation of trifluoroacetylated proteins (CF_3CO-proteins). CF_3CO-proteins are formed in all exposed individuals. The majority of individuals immunologically tolerates CF_3CO-poteins. However, a small subset of susceptible individuals develops halothane hepatitis, which involves an immune reaction towards CF_3CO-proteins. Our work focuses on mechanisms that lead to a break of tolerance towards the CF_3CO-motif in these patients. Such tolerance is likely to exist due to the constitutive presence of lipoic acid, one of the prosthetic groups of 2-oxoacid dehydrogenase complexes in the repertoire of self. Lipoic acid structurally and immunochemically mimics the CF_3CO-motif. In comparison to normal individuals, halothane hepatitis patients appear to express less or aberrant forms of the lipoic acid bearing proteins. Investigations of the human gene coding for the E2-PDC by Northern blot- and PCR- techniques indicate the occurrence of multiple mRNA species, suggesting the expression of variant forms of the E2-PDC. Screening the multiple forms of genes coding for the E2-PDC should allow to identify genetic aberrancies that might distinguish susceptible individuals from healthy controls.

S16-28

RECOMBINANT IMMUNOGLOBULIN A EXPRESSED IN A MOUSE MAMMARY GLAND CELL LINE

L. Rindisbacher*, J. Berdoz#, N. Jeanguenat#, B. Corthésy*, and J.-P. Kraehenbuhl#
*Institut de Biologie animale, Université de Lausanne
#Institut Suisse de Recherches Expérimentales sur le Cancer

Orally or nasally administered monoclonal immunoglobulin A (IgA) antibodies are able to protect mucosal surfaces against invasion by a variety of different pathogens. Our goal is to evaluate heterologous expression systems for the production of large quantities of recombinant IgA for passive immunoprotection. Expression of heterologous genes in the lactating mammary gland of transgenic livestock and secretion of the recombinant product into the milk offers an attractive approach for mass production of proteins for medical applications.

We want to test whether functional antibodies can be produced by the mammary gland epithelium. Therefore, we are currently establishing mouse mammary gland cell lines stably transfected with the heavy- and light chain genes of a recombinant antibody. The genes were put under control of either the strong, constitutive CMV promoter or the inducible, lactation specific promoter for bovine αS1-casein. We will test whether functional antibody is vectorially secreted into the medium.

S16-29

The phenotype of malignantly transformed mammary epithelial cells can be largely modulated in collagen matrices

Claude Rudaz, Christine Meyer and Ernst Reichmann
Swiss Institute For Experimental Cancer Research (ISREC)

We are studying the effects of certain tumor-relevant oncoproteins on polarized mammary epithelial cells in culture. As one model-oncogene we have introduced the v-Ha-ras gene into non-tumorigenic mouse mammary epithelial cells. Subsequently, both normal and Ras-expressing mammary epithelial cells have been plated within reconstituted collagen type I gels. Taking advantage of this in vitro system and adopting it to the epithelial cell clones employed, we managed to let normal, untransformed cells develop into three-dimensional organotypic structures. These structures largely resembled distinct stages of mammary gland development, differentiating into ducts and end buds and even into alveoli-like structures, in all of which the formation of true lumina was observed. In contrast, Ras-transformed mammary epithelial cells, when cultured in serum-free conditions, developed into cystic, adenocarcinoma-like structures which exhibited large lumina that were lined by polarized epithelial cells. Upon the addition of fetal calf serum the same cells developed into solid ridges consisting of invasively growing unpolarized spindle-shaped cells. These fibroblastoid cells had lost certain epithelial properties, such as tight junctions and uvomorulin/E-cadherin and had gained fibroblastic markers, like vimentin and collagen type I. In addition these cells were higly invasive both in collagen gels and in the chicken heart assay. Our findings provide evidence that dependent on the culture conditions, highly tumorigenic, v-Ha-ras transformed mammary epithelial cells have the potency to develop into completely distict phenotypes.

S16-30

A RECOMBINANT VACCINIA VIRUS EXPRESSING THE MOUSE MAMMARY TUMOR VIRUS ORF PROTEIN INDUCES A SUPERANTIGEN RESPONSE IN INFECTED MICE.

C. Krummenacher[1], H. Acha-Orbea[2], and H. Diggelmann[1]
[1] Institute for Microbiology, University of Lausanne, Ch. des Boveresses 155, 1066 Epalinges
[2] Ludwig Institute for Cancer Research, Lausanne Branch, Ch. des Boveresses 155, 1066 Epalinges

The Mouse Mammary Tumor Virus (MMTV) superantigen (SAg) is encoded by the orf gene in the 3' long terminal repeat of the proviral DNA.

We produced recombinant vaccinia viruses expressing the complete or a truncated ORF protein starting at the fifth in-frame ATG (ORF5) of MMTV GR. The superantigen of MMTV GR acts specifically on CD4+ T-cells bearing the Vβ14 T cell receptor. We studied the SAg activity of both recombinants after infection of BALB/c mice. The CD4 T-cell repertoire in the draining popliteal lymph node was analysed after injection of the virus in the footpad.

In mice infected with the complete orf recombinant vaccinia virus we observed a strong, rapid and specific stimulation of Vβ14+ CD4+ T-lymphocytes in the local lymph node in response to the superantigen. In contrast, the infection with the ORF5 recombinant does not generate such a response. The proliferative response was qualitatively and quantitativaly different from the one observed after MMTV infection. By PCR analysis of the lymph nodes we detected viral sequences preferentially in adherent cells, suggesting that members of the macrophage-monocyte lineage are the major targets for infection by the recombinant vaccinia viruses.

S16-31

Targeting of the mouse mammary tumor virus receptor using binding studies with recombinant envelop-glycoproteins

F. Luthi[1], H. Acha-Orbea[2] and H. Diggelmann[1]
[1] Institute for Microbiology, University of Lausanne, Ch. des boveresses 155, Ch-1066 Epalinges, Switzerland
[2] Institut of Biochemistry, University of Lausanne, Ch. des boveresses 155, Ch-1066 Epalinges, Switzerland

Mouse mammary tumor virus (MMTV) is produced in the mammary gland of infected females and transmitted to suckling new-borns through the milk. The passage of MMTV from the gut to the mammary gland is only poorly understood. Recently it has been reported that the virus is capable to penetrate the epithelium of the gut but replication seem to occur occur only in the Peyer's patches. B lymphocytes seem to be the primary targets for MMTV infection resulting in the expression and presentation of the viral superantigen. Infected B and T lymphocytes might carry the virus to the final target organ, the mammary gland. The presence of a single receptor on all target cells can be postulated; alternatively different surface molecules might be involved in viral uptake.
In order to define the nature of a potential receptor for MMTV, we expressed the envelop-glycoprotein (gp52) in bacteria and from a recombinant baculovirus in insect cells. Binding studies have been performed with both proteins coupled to fluorescent latex beads on lymphocytes. Preliminary results show a clear binding to B cells but not to T cells. Cross-linking experiments and affinity columns should allow further caracterisation of a potential receptor for MMTV.

S16-32

THROMBIN RECEPTOR IMMUNOREACTIVITY IN THE RAT NERVOUS SYSTEM

S. Niclou, H.S. Suidan, S.R. Stone* and D. Monard
Friedrich Miescher-Institut, P.O. Box 2543, 4002 Basel, Switzerland.
*Department of Haematology, MRC Centre, Cambridge, UK.

The serine protease thrombin dramatically affects the morphology of cultured neuronal and astroglial cells via cleavage-induced activation of a G-protein coupled receptor. This receptor is hypothesized to play a role in establishing connectivity patterns in neural tissues. In the present study the distribution of the thrombin receptor in the nervous system is reported. By Northern analysis, the level of receptor mRNA was found to be higher in the embryonic and early postnatal brain as compared to the adult. These results were corroborated by immunohistochemical data showing that the immunoreactivity was widespread in the embryonic nervous system and in the P7 brain, whereas in the adult it was confined to particular structures. These include the olfactory system, the ventral tegmental area and hindbrain tegmentum, the Purkinje cells and cells of the white matter. Neuronal as well as glial cells were stained. The present results lay the basis needed to begin to understand the role played by the thrombin receptor in the developing and adult nervous system.

S16-33

HEMATOPOIETIC RESCUE BY MELATONIN-INDUCED ENDOGENOUS IL4 AND GM-CSF.

Georges JM Maestroni and Ario Conti
Center for Experimental Pathology, Istituto Cantonale di Patologia, Locarno.

In previous work we have shown that the pineal neurohormone melatonin can augment the immune response and correct immunodeficiency states. Recently we found that when administered together with cancer chemotherapy compounds, melatonin did not influence tumor growth but selectively counteracted hematopoietic toxicity without interfering with their anti-cancer action. Melatonin proved to counteract apoptosis in bone marrow cells incubated with etoposide. The effect of melatonin was neutralized by anti-GM-CSF monoclonal antibodies. In presence of suboptimal concentrations of GM-CSF, melatonin was able to augment the number of GM-CFU. When bone marrow from athymic, T-cell-deficient mice or CD4+, T-helper cells-depleted marrow was used, melatonin did not exert any effect. Further studies revealed that melatonin acts on Th2 cells inducing, both at physiological and pharmacological concentrations, the release of IL4. In turn, IL4 stimulates the production of GM-CSF by adherent bone marrow cells. Due to the well known lack of toxicity of melatonin, our finding may thus have straightforward clinical applications.

S16-34

HEMATOPOIETIC MODULATION VIA a-ADRENERGIC RECEPTORS

Georges JM Maestroni, Mauro Togni, Valeria Covacci, Ario Conti
Centre for Experimental Pathology, Istituto Cantonale di Patologia, 6604 Locarno, Switzerland

We recently demonstrated that adrenergic agents can affect hematopoiesis after syngenic bone marrow transplantation in mice. In particular, administration in vivo of α1-adrenergic antagonist prazosin was shown to increase myelopoiesis. Furthermore noradrenaline protected mice which were injected with a lethal dose of carboplatin. In vitro we observed an inhibition of CFU-GM in presence of noradrenaline. Furthermore, we found that 3H-Prazosin binds specifically to bone marrow cell membranes and intact bone marrow cells. Scatchard analysis of saturation curves revealed the presence of two binding sites. A Kd of 0.98 ± 0.32 nM and B max of 5 ± 2.9 fmol/2x10^6 cells characterised the higher affinity site, while the lower affinity site displayed a Kd of 55.9 ± 8.2 nM and a B max of 44 ± 7.7 fmol/2x10^6 cells. By different separation methods we fractionated bone marrow cells in various subpopulations, resulting in separation of the two binding sites. The low affinity binding site seems present on Mac1+B220- cells, while the high affinity binding site is beared by loosely adherent Mac1-B220+IgM- cells. Our findings suggest that the sympathetic, noradrenergic influence on pre B cells constitutes an important regulator of hematopoiesis which might be used to prevent the myeloablative effect of anti-cancer treatments.

S16-35

AN IN VITRO MODEL FOR MYELOTOXICITY BASED ON HUMAN UMBILICAL CORD BLOOD PROGENITORS CELLS TO TEST NEW ANTI-TUMOR AGENTS.

Bosshard G., Soldati G., Sessa C., D'Incalci M., Ghielmini M. and Cavalli F.
Dpt. of Oncology, "La Carità" Hospital, 6600 Locarno and Istituto Mario Negri, Milano, Italy.

Introduction. Myelotoxicity is the most common side effect of anticancer drugs. Eventhough the clinical features of myelosuppression of the currently used antitumor agents are well characterized, no data are available on the relationship between the mechanism of action and the cellular damage induced on the hematopoietic progenitor pool. The aim of this study is to set up an in vitro test to better understand the effects of new antitumor agents on the hematopoietic progenitors and to dissect these effects on the different sub-populations present in human umbilical cord blood (hCB). In particular we are testing the effects of Tallimustine (FCE296), a new DNA minor-groove binder, actually tested in clinical phase I trial in our Department.
Materials. hCB is collected, separated by gradient centrifugation (Ficoll), adherent cell depleted and cultured on standard clonogenic assays in methylcellulose after having exposed to different concentrations of FCE296 for 1 hr. or continously. CFU-GM, BFU-e and CFU-e clones are counted at 7 and 14 days.
Results. Preliminary results show that the hCB progenitors are comparable to bone marrow (BM) in terms of cell survival. Continous or 1 hr exposure to the anti tumor agent showed that the former was more myelotoxic than the latter (ID50(1hr)=140 ng/ml vs ID50(cont.)=30 ng/ml in GM-CFU counts). The same pattern was observed with BFU-e and CFU-e. Overall, all the CFCs behave the same way except for CFU-e at 14 days which showed to be more resistant to the drug (60% survival compared to 10-20% survival for the other CFCs). The possible predictability of the test is suggested by the fact that the ID70 of the GM-CFU is 160 ng/ml, the same concentration observed in the serum of patients after 1 hour from treatment with FCE296 at the MTD.
Conclusions. This test could replace previous tests based on BM cells, which are more difficult to obtain. Furthermore, the 1 hr exposure seems to approximate the in vivo toxicity. Finally, it is possible to differentiate the effects on different sub-populations of progenitors.

S16-36

SENILE CATARACT IS DUE TO FORMATION OF A QUINONE IN THE HUMAN LENS

X. D. Martin & H. Z. Malina

Laboratoire d' Ophtalmologie Expérimentale,
Institut Universitaire de Pathologie, CH-1011 Lausanne

3-hydroxykynurenine is a UV filter in normal human lenses. In this study we observed that in the cataractous lens 3-hydroxykynurenine is deaminated. The enzyme responsible for this reaction is kynurenine aminotransferase (KAT). The KAT activity was totally absent in the lens of young subjects (below 30 years of age). In lenses of about 50 years of age, KAT activity reached up to 980 nmol/mg protein/hour. In cataractous lenses, KAT activity found in the transparent cortex varied between 22 and 485 nmol/mg protein/hour. The xanthurenic acid formed was then oxidized giving oxo-xanthurenic acid (OXA) and di-oxo-xanthurenic acid (DOXA). The later compound is a naphthalene like quinone. The induction of KAT seems to be the first event leading to senile cataract formation.

Swiss National Science Foundation 32-36058.92.

S16-37

EFFECT OF RESPIRATORY MUSCLES TRAINING ON AIRWAY RESISTANCE AND ON VENTILATION DURING ENDURANCE EXERCISE.

Kohl, J., Cardenas, M., Brandenberger, M., Boutellier, U., and Koller E.A. Physiologisches Institut der Universität, CH-8057 Zürich

Respiratory training prolonged endurance performance at anaerobic threshold both in sedentary and in trained subjects, presumably by reducing the hyperventilation at the given work load. The purpose of the present study was to investigate the effect of exercise-induced hyperventilation and hypocapnia on airway resistance and to answer the question whether the eventual hypocapnic bronchoconstriction prior to respiratory training might be the factor limiting endurance. Nine healthy volunteers of either sexes participated in the study. Cycling and breathing endurance tests as well as body plethysmographic measurements of pre- and post-exercise airway resistance (Raw) were carried out before and after a four weeks lasting respiratory training. In one of the two experimental sessions before the respiratory training carbon dioxide was added to the inspired air to keep its end-tidal concentration at 5.4% to avoid hypocapnia. The respiratory training was successful as it increased significantly the respiratory endurance in all subjects. Cycling endurance correlated significantly with minute ventilation when all three tests were compared: All subjects performed the longer the lower ventilation was. Neither the pre- nor the post-exercise values of Raw were significantly changed after the respiratory training, and there was no significant correlation between end tidal carbon dioxide and Raw. In conclusion: Exercise-induced hyperventilation has no effect on Raw, and some other mechanisms than lower Raw are responsible for prolongation of cycling endurance which is associated with the reduction of hyperventilation as observed after respiratory training.

S16-38

USE OF A NEURAL NETWORK TECHNIQUE FOR ASSESSING THE SPEED AND INCLINE OF WALKING IN MAN BY MEANS OF 3-DIMENSIONAL ACCELEROMETRY

Schutz Y., Aminian K.*, Jéquier E., Robert Ph.*, Institut de Physiologie de l'Université, Bugnon 7, 1005 Lausanne et *Laboratoire de Métrologie, EPFL

The purpose of the study was to develop a device - based on body acceleration - which permits the measurement of the speed, the distance and the incline of walking. A portable data logger was designed to record body accelerations in 3 dimensions (forward, vertical and lateral). Two neural networks were designed to recognize each acceleration pattern. They were first individually trained by known patterns of speed and elevation of treadmill walking. Six subjects performed first a treadmill walking followed by a self-pace walking on an outdoor test circuit with various inclines. The average uncertainty of the prediction of incline was around 1% slope. The individual differences between the real distance covered and that predicted by neural network from accelerometry ranged from 2 - 4%. To the best of our knowledge, these results constitute the first objective assessment in man of speed, incline and distance of walking in free living conditions.

S16-39

Quantification of Myocardial Microvasculature by Laser Scanning Confocal Microscopy and Stereology *in vivo*

Johannes C. Fleischhauer, Neil Roberts*, Peter Lipp, Ernst Niggli, Lilly Lehmann, André G. Kléber, University of Bern, Bern, Switzerland and *University of Liverpool, Liverpool, United Kingdom

To assess the volume, branching pattern and length of the myocardial microvasculature and capillary diameter *in vivo*, we used laser scanning confocal microscopy in the arterially-perfused rabbit papillary muscle (Tyrode's solution, dextran [40 g/l], 10 % newborn calf serum). Butanedione Monoxime (20 mM) was added to reduce mechanical activity. The perfused cylindrical muscle (diameter 0.8-1.2 mm) was placed on the stage of an inverted confocal microscope and optical sections were obtained at 1μm intervals from the surface to a depth of 71 μm. After staining cell membranes with di-8-ANEPPS (1μM), optical sectioning revealed an endothelial cell layer (8μm) and a dense capillary network appearing at a depth of 10μm. A rectangular tissue block of 307x205x48μm was analyzed using modern design based stereological techniques. In this block, total vascular volume fraction (including vessel walls) was 23.8 %, total capillary length was 4078 mm/mm^3 and the number of vessel junctions in capillaries 54'000/mm^3. Average outer vessel diameter was 8.6μm, inner diameter 4.9μm. Conclusion: The relatively large volume fraction of microvessels is explained by the inclusion of vessel wall thickness. Confocal microscopy provides an accurate 3-dimensional reconstruction of the capillary tree *in vivo*.

S16-40

VOLUME CHANGES IN FOREARM AND LOWER LIMB DURING ACUTE HYPOBARIC HYPOXIA.

Schirlo, C., Bub, A., Reize, C., Bührer, A., Kohl, J., and Koller, E.A. Institute of Physiology, University of Zurich, CH-8057 Zurich, Switzerland

Acute hypobaric hypoxia (AHH) leads to hemoconcentration and a decrease in plasma volume (PV). In order to investigate the role of fluid shifts from peripheral to central compartments during AHH the changes in lower limb (LV) and forearm volumes (FV) were plethysmographically measured in 10 healthy volunteers exposed to graded AHH in a hypobaric chamber. Total altitude exposure period of the stepwise ascent and descent (450m-2500m-3500m-4500m-2500m-450m) lasted 2 hrs. During ascent a decrease both in LV and FV of -0.52±0.39 ml/100ml (mean±SD) and -0.65±0.32 ml/100ml respectively was found. However, during descent LV shows a further small, but not significant decrease (-0.02±0.11ml/100ml), whereas FV tends to increase slightly (+0.03±0.13 ml/100ml). During the whole AHH exposure PV decreased (-4.66±2.95%) and urine volume increased (0.84±0.41 to 3.29±1.43 ml/min). We conclude that the decreases in LV and FV under AHH reflect a blood volume shift from peripheral to central compartments and that the decrease in PV might be due to the elevated diuresis and not to an enhanced filtration of intravascular fluid into tissue.

S16-41

VALIDATION OF THE STRÖMBERG-MIKA CELL MOTION ANALYZER

G. Togni, G. Medici, A. Piffareti-Yanez, F. Keller, M. Balerna
Gynaecological Endocrinology Unit, "La Carità" Hospital, Locarno (Switzerland)

Introduction

Computer assisted semen analysis (CASA) systems have received much attention during the last years. From the studies published until now, these systems have shown positive and negative characteristics. The present study was aimed at evaluating the performance of the Strömberg-Mika system (SM-CMA).

Materials and methods

Fresh semen samples from patients consulting our unit for infertility were used. Sperm concentration and motility were assessed by changing the IMO/LOC, AREAMIN and FRMAX variables. The experimental data were also used to assess the accuracy, precision and specificity of the system. Finally, the SM-CMA measurements were compared to those obtained by subjective and semi-automated assessment (MEP).

Results and conclusion

The internal precision of the SM system was demonstrated by re-measuring 10 times the concentration of 10 samples (mean CV%=0.019). The within-series precision assessed by measuring 8 different positions of the Makler chamber increases as function of the sperm concentration, furthermore, the variation of the measurement was lowered when the video image was manually corrected. The rough CV%s measured on a fixed position of the Makler chamber are lower than those calculated by varying the position. The manual correction option enhanced the precision (CV lower than 5% even at low concentrations). The specificity was assessed by comparing the corrected and uncorrected measurements on both fixed and varying position; the CV% was found to be higher at low sperm counts. The accuracy was assessed by comparing the SM-system uncorrected and corrected sperm counts with those obtained by subjective or by manually evaluating the video image. Uncorrected measurements were constantly and significantly lower than those obtained by the three previous mentioned approaches.

S16-42

HEART TRANSPLANTED CHILDREN (HTR-C): IS THERE ANY EVIDENCE FOR FUNCTIONAL CARDIAC REINNERVATION?

C. Marconi, M. Marzorati, M. Conti, B. Grassi, P. Ferrazzi, R. Fiocchi, E. Cohen-Laroque, and P. Cerretelli. I.T.B.A.-C.N.R., Milano (I); Dept. of Cardiovasc. Surgery, Bergamo Hosp. (I) and Dept. of Physiology, Univ. of Geneva, (CH).

To assess whether transplantation in pediatric age is followed by cardiac reinnervation, heart rate (HR) of 10 HTR-C, aged 13±3 yr. (mean±SD), and 6 adult HTR (HTR-A), aged 31±7 yr., was recorded at rest and during graded cycling exercise 3 yr after surgery. At rest, HR of HTR-C was 99±11 b/min (90±11 in HTR-A), with evidence of variability (as shown by spectral analysis). At peak exercise, HR was 169±24 (HTR-C) and 163±14 (HTR-A), higher than usually reported for adult HTR. The HR response at the onset of a submaximal constant load was faster in both HTR-C and HTR-A than previously reported. CONCLUSIONS. There is some evidence of reinnervation in HTR-C. However, cardiac response to exercise may be affected by other factors (e.g. to explain the fast HR readjustment of the present HTR-A), such as type of cardiopathy before transplantation, deterioration of skeletal muscles, sensitivity to catecholamines, immunosuppressive treatment and, possibly, surgical technique (Part. supp. by FNRS 32-40397, 94/1 and CNR Target Project BTBS).

S16-43

STEREOLOGIC ESTIMATION OF AIRWAY MACROPHAGE NUMBERS IN LAVAGED HAMSTER LUNGS

Serra, A.L., Geiser, M., Baumann, M., Im Hof, V. and Gehr, P., Department of Anatomy, University of Berne, CH-3000 Bern 9

Macrophages play a central role in the defense of the respiratory tract against deposited particles. Functional and morphologic investigations of alveolar and airway macrophages have been performed *in vitro* using bronchoalveolar lavage or lavage of the extrapulmonary airways only. So far, no morphometric studies existed which showed the effectiveness of macrophage recovery by this technique. Therefore, we lavaged the lungs of four hamsters and fixed the lungs thereafter by intravascular perfusion. The number of macrophages in the intrapulmonary conducting airways was estimated with an unbiased stereologic technique, the fractionator, and compared to the number of macrophages in the airways of four hamsters, whose lungs had not been lavaged prior to fixation. Additionally, qualitative ultrastructural analysis of the airway wall was performed. This *in situ* study revealed (1) that 40 % of the airway macrophages were not removed by bronchoalveolar lavage; (2) that about 5 % of all macrophages in the bronchoalveolar lavage fluid were airway macrophages, and (3) that there occur ultrastructural changes in the airway wall after lavage. Apparently there exists a population of airway macrophages that resists lavage by a yet unexplained enhanced adhesiveness to the epithelium. We expect them to be functionally and morphologically different from those airway macrophages which are easily removed by lavage.

Synaptic transmission and molecular neurobiology

S17-01

NEUROENDOCRINE PROPERTIES OF PITUITARY CELLS IN RAT ORGANOTYPIC SLICE CULTURES: A NEW APPROACH FOR STUDYING CELL-TO-CELL COMMUNICATION

Nathalie C. Guérineau, R. Anne McKinney, Dominique Debanne, Lotty Rietschin and Beat H. Gähwiler *Brain Research Institute, CH-8029 Zürich*

Neuroendocrine and electrical properties of anterior pituitary cells were investigated in organotypic slice cultures prepared from 6-7 day-old rats, as described by Gähwiler (1981). After 30-40 days *in vitro*, pituitary cells maintain the characteristics of endocrine cells, including secretory granules as seen by electron microscopy and the presence of hypophysial hormones (prolactin PRL) as detected by immunofluorescence. Electrophysiological properties were investigated with microelectrodes. Pituitary cells had a membrane input resistance of ~250 MΩ, the membrane resting potential was ~-50 mV and action potentials were found in ~50% of cells. As PRL-secreting cells were present, the effects of thyrotropin-releasing hormone (TRH), a PRL release-stimulating neuropeptide, were examined using the patch-clamp technique. Bath-application of 100 nM TRH triggered an outward current (25 ± 8.6 pA, HP=-40 mV) associated with an increase in membrane conductance. Of particular interest, was the observation that many cells were coupled through gap junctions as shown by injecting cells with Lucifer Yellow (4%). In conclusion, these data confirm the viability of organotypic slice cultures of pituitary gland, and demonstrate that the characteristic properties of this tissue are conserved. This provides us with a new preparation that is especially suitable for the study of the mechanisms regulating cell-to-cell communication under conditions of intact cellular organization.

S17-02

DEPRESSION OF EVOKED IPSPs BY PRESYNAPTIC $GABA_B$ RECEPTORS IN RAT CEREBELLUM IN VITRO

By D. Mouginot and B. H. Gähwiler
Brain Research Institute, August Forel-Str. 1, 8029 ZURICH, Switzerland

In cerebellar slice cultures, Purkinje cells (PCs) establish inhibitory connections with deep cerebellar nuclei (DCN) neurons *de novo*. We studied the depression of inhibitory synaptic transmission by baclofen, a selective $GABA_B$ receptor agonist.

In the presence of excitatory amino acid receptor antagonists (CNQX and D-APV), field stimulation within the PC layer induced monosynaptic $GABA_A$ receptor-mediated IPSPs in DCN neurons recorded with sharp electrodes. In DCN neurons impaled with electrodes filled with a solution containing QX 314 (50 mM) which blocked the postsynaptic $GABA_B$ response, bath application of baclofen (10 μM) depressed evoked IPSPs by 37 ± 19 %. Furthermore, baclofen (100 μM) did not modify the amplitude and the slope of postsynaptic muscimol-induced responses, indicating that presynaptic $GABA_B$ receptors are responsible for the depression of the inhibitory synaptic potentials. The $GABA_B$ receptors appear to be located on synaptic terminals of PCs because (i) the reduction in amplitude of evoked IPSPs was still observed when the stimulating electrode was placed within the fiber bundle connecting PCs to DCN neurons. (ii) bath application of baclofen (100 μM) had no detectable effect on PCs, in particular, potassium currents were not induced (iii) Under conditions of synaptic blockade, the rate of spontaneous discharge of PCs (12 ± 7 Hz) was not changed by baclofen.

S17-03

SUBFORNICAL ORGAN OF RAT, MOUSE, AND MONGOLIAN GERBIL IS A SOURCE OF NITRIC OXIDE.

Krstic R., Nicolas D., Novier A., Institut d'Histologie et d'Embryologie, Rue du Bugnon 9, 1005 Lausanne.

Nitric oxide synthase (NOS) is a calmodulin-dependent enzyme that converts L-arginine into nitric oxide (NO) and citrulline. At present, NO is considered as a gazeous neuromodulator with an important role in both central and peripheral nervous systems. The subfornical organ (SFO) is a small well-vascularized neuron-glial circumventricular organ located below the anterior commissure dorsal to Monro's foramen, probably involved in sensory functions within autonomic circuits and playing a role in body fluid balance. Very little is known about NOS presence in the SFO. Only Jurzak et al (Brain Res. 662: 198, 1994) found a high concentration of NOS in small SFO-neurons of the rat.

Using NADPH-diaphorase histochemistry to localize NOS, we confirmed the findings of Jurzak et al (1994) in SFO of rat and also demonstrated a high activity of this enzyme in SFO neurons in mouse and Mongolian gerbil (Merio unguiculatus). In comparison with the neighboring subcommissural organ, where NOS content was variable and species dependent, the NOS concentration in SFO neurons was constantly elevated. Therefore we consider the SFO of the above mentioned animals as a source of NO which could diffuse not only among adjacent neurons, but also into the cerebrospinal fluid.

S17-04

AMPA-RECEPTOR CHANGES IN POSTNATAL CEREBELLUM

Bi, X.-N. and Streit, P., Inst. f. Hirnforschung, Univ. Zürich, 8029 Zürich.

The patterns of immunoreactivity were studied in rat vermis from postnatal day 1 (P1) to P78 using antibodies to AMPA-receptor subunits GluR1, -2/3(4c) and -4. Throughout this period, antibodies to GluR1 and -4 stained Golgi epithelial cells most intensely, while Purkinje cells were the most heavily immunoreactive elements with anti-GluR2/3(4c) antibodies. P1 - P12: Labeling of these two cell types was strongest in parts of cerebellar folia most actively involved in processes of growth and differentiation. P15 - P22: Their immunoreactivity was homogeneous in intensity throughout all lobules. P24 - P36: Striking heterogeneities were observed. Certain Golgi epithelial cells as well as dendritic trees of some Purkinje cells were more weakly labeled or even unstained. These elements were arranged in patches or as more or less densely arranged single components between intensely immunoreactive neighbors. P45 - P78: The staining pattern became more and more homogeneous again with the most ventral parts being latest to recover. - The changes in AMPA-receptor expression lasting until adulthood was reached might have functional implications in the maturation of cerebellar circuitry and/or in cerebellar information processing.

S17-05

GLUTAMATE UPTAKE INTO ASTROCYTES STIMULATES AEROBIC GLYCOLYSIS: A MECHANISM COUPLING NEURONAL ACTIVITY TO GLUCOSE UTILIZATION. L. Pellerin and P.J. Magistretti. Institut de Physiologie, Université de Lausanne.

Astrocyte end-feet surround intraparenchymal microvessels and represent therefore the first cellular barrier for glucose entering the brain. As such, they are a likely site of prevalent glucose uptake. We have recently demonstrated that glutamate (Glut), the main excitatory neurotransmitter, stimulates in a concentration-dependent manner 2-DG uptake and phosphorylation by astrocytes in primary culture (PNAS 91:10625-10629). The effect is not receptor-mediated since it is not reproduced by Glut agonists nor prevented by antagonists. Rather, it is mediated by one of the recently cloned Glut transporter, since the effect is blocked by either THA or L-CCG III, two inhibitors of Glut transporters. Accordingly, replacement of Na^+ in the medium by choline also prevents the effect of Glut, consistent with the Na^+-dependent nature of Glut transport. Ouabain, an inhibitor of Na^+/K^+ ATPase, completely blocked the effect of Glut. Concomitant to the stimulation of glucose uptake, Glut causes a concentration-dependent increase in lactate efflux. This effect is also blocked by THA. These observations suggest that glutamate uptake is coupled to aerobic glycolysis in astrocytes. In addition, since glutamate release occurs following the modality-specific activation of a brain region, the glutamate-evoked uptake of glucose into astrocytes provides a simple mechanism to couple neuronal activity to energy metabolism. These data also suggest that modality-specific activation visualized using 2DG-based autoradiography or PET may primarily reflect glutamate-mediated uptake of 2DG into astrocytes.

S17-06

2-DEOXYGLUCOSE UPTAKE AND *C-FOS* EXPRESSION, TWO MARKERS OF NEURAL ACTIVATION *IN VIVO*, ARE DIFFERENTIALLY STIMULATED BY GLUTAMATE IN ASTROCYTES AND NEURONS *IN VITRO*

L. PELLERIN, J.-L. MARTIN AND P.J. MAGISTRETTI. INSTITUT DE PHYSIOLOGIE, UNIVERSITÉ DE LAUSANNE.

We have previously shown that glutamate increases 2-deoxyglucose (2DG) uptake and phosphorylation in astrocytes by a mechanism involving a Na^+-dependent glutamate transporter (LP & PJM, PNAS 91:10625-10629). In contrast, no effect of glutamate was observed in cultured cerebral cortical neurons. In an attempt to elucidate the basis of this difference, we have characterized 2DG uptake (with ^{3}H-2DG) and glutamate uptake (with ^{3}H-D-Asp) in both cell types. The Vmax for ^{3}H-2DG uptake in neurons and astrocytes were respectively (in nmol/mg prot/min): 6.3 and 22.3. For ^{3}H-D-Asp uptake, in neurons and astrocytes Vmax were respectively (in nmol/mg prot/min): 0.74 and 57.4. These data clearly indicate a prevalent glucose and glutamate uptake into astrocytes. Since 2DG uptake is a technique used *in vivo* to map regional brain activation, we examined the effect of glutamate on *c-fos* expression, another marker used for mapping activation. Glutamate at 100 μM induced a fivefold increase of *c-fos* expression in neurons while the same treatment had no effect on astrocytes. Taken together, these results suggest that, while *c-fos* expression appears to be a specific marker for neuronal activation, 2DG uptake, although directly related to neuronal activation, is localized to astrocytes.

S17-07

NORADRENALINE (NA) ACTIVATES A K^+ CONDUCTANCE AND INHIBITS A GLUTAMATE MEDIATED TRANSMISSION IN THE MOUSE ENTORHINAL CORTEX (EC) *IN VITRO*

E. PRALONG, R.S.G. JONES* AND P.J. MAGISTRETTI. INSTITUT DE PHYSIOLOGIE, UNIVERSITÉ DE LAUSANNE AND *DEPARTMENT OF PHARMACOLOGY, OXFORD UNIVERSITY

NA via α_2 receptors hyperpolarizes neurones and reduces global synaptic transmission in layer II of EC (E. Pralong and P.J. Magistretti, Neurosci. Lett.1994 179, 145-148). We have now investigated the ionic basis of this action of NA in the EC. NA (50 μM) hyperpolarized 55/83, depolarized 7 and did not affect 21, layer II EC neurones. In the presence of 1 μM tetrodotoxin, NA (50 μM) reduced the membrane resistance by 18.9 ± 1.6 % and hyperpolarized EC cells by -3.8 ± 0.4 mV (results ± sem, n=13). Equilibrium potential of the NA induced outward current varied with log $[K^+]_{out}$. When synaptic responses were evoked in EC layer II cells, NA reduced the pharmacologically isolated NMDA and AMPA mediated EPSPs: 50 μM NA reduced NMDA response to 19.1 ± 2.5 % (n=12) and AMPA response to 35.7 ± 2.8 % (n=7) of the control response. NA (50 μM) did not affect $GABA_A$ reponse (91.1 ± 7 %, n=6). Whole cell patch experiments performed in rat EC confirm the specific action of NA on glutamatergic currents. These results strongly suggest a post-synaptic activation by NA of α_2 receptors coupled via a G-protein to a K^+ conductance as well as a reduction of glutamate-mediated synaptic transmission in EC. 31-40'585.94.

S17-08

Identification by differential display of glutamate-modulated mRNAs during development of cultured cortical neurons.

G. Pellegri, P.J. Magistretti and J.-L. Martin. Institut de Physiologie, Université de Lausanne, Switzerland.

Chronic exposure of cultured cortical neurons for six days to the NMDA receptor antagonist MK-801 totally abolishes *c-fos* expression, therefore suggesting that the basal expression of *c-fos* is due to endogenous glutamate present in the medium. In view of this observation and of the role played by the Fos protein as a transcription factor, we set out to identify late genes whose expression is modulated by endogenous glutamate acting on NMDA receptors during development of cortical neurons. To this aim, we have set up the technique of differential display, which allows to identify by PCR, differentially expressed mRNAs. Using this technique we have observed several differences in the pattern of amplified mRNAs in control (stimulated by endogenous glutamate) and MK-801 treated cultures. We are presently characterizing by Northern blot and DNA sequencing these glutamate-modulated mRNAs. In this context we have recently observed that chronic exposure of cortical neurons to MK-801 markedly inhibits brain-derived neurotrophic factor (BDNF) mRNA expression, therefore lending further support to the role played by glutamate on the development of cortical neurons.

S17-09

VIP and PACAP potentiate *c-fos* expression induced by glutamate in cultured cortical neurons.

J.-L. Martin, D. Gasser and P.J. Magistretti. Institut de Physiologie, Université de Lausanne, Switzerland.

VIP increases cAMP levels and stimulates *c-fos* expression in primary cultures of cortical neurons. However, VIP induction of *c-fos* expression is completely inhibited by the non-competitive NMDA receptor antagonist MK-801, therefore indicating that VIP stimulates *c-fos* expression in a glutamate-dependent manner. A similar effect was observed with Pituitary Adenylate Cyclase-Activating Polypeptide27 (PACAP27). At the intracellular level, co-activation of protein kinases A and C mediates the glutamate-dependent stimulation of *c-fos* expression evoked by VIP, since either H-89 or staurosporin inhibit the effect of VIP as well as that of glutamate. These results point to a "biochemical AND gate" mechanism, which implies the obligatory activation of both protein kinases A and C in the transduction of *c-fos* expression. These results provide evidence that VIP and PACAP27 potentiate the effect of glutamate, the principal effector, on *c-fos* expression suggesting that both peptides can increase the "throughput" or "strength" of glutamate-containing circuits in the cerebral cortex.

S17-10

DISTINCT DEVELOPMENTAL EXPRESSION OF THE FIVE NMDA RECEPTOR SUBUNITS NR1 AND 2A-D INVESTIGATED WITH SUBUNIT SPECIFIC ANTISERA IN RAT BRAIN

A. Wenzel, D. Benke, J.M. Fritschy and H. Mohler
Inst. of Pharmacology, ETH and University of Zurich, Winterthurerstr. 190, 8057 Zurich

The regional distribution of NMDA receptor subunits NR1 and NR2A-D was investigated on the protein level using subunit-specific antisera. Western blot analysis revealed an apparent molecular size ranging between 115 (NR1) and 175 KDa (NR2B). All subunits displayed distinct, though overlapping, patterns of distribution in adult rat brain, as analyzed on brain sections blotted onto nitrocellulose for immunostaining. At birth only the NR1-, NR2B- and NR2D subunits were detected. Around one week after birth, the NR2A and NR2C immunoreactivity appeared, pointing to the emergence of additional NMDA receptor subtypes during postnatal development.

S17-11

EFFECT OF EXCITATORY AMINO ACID RECEPTOR AGONISTS ON THE RELEASE OF THE NITRIC OXIDE PRECURSOR ARGININE FROM RAT CEREBELLAR SLICES.

G. Grima, B.Benz and K.Q. Do. Brain Research Institute, University of Zürich, 8029 Zürich, Switzerland.

Arginine (Arg), the nitric oxide (NO) precursor, has been found to be released following stimulation of the white matter in cerebellar slices (Hansel et al., 1992) and stimulation of sensory afferents in rat thalamus in vivo (Do et al., 1994). NO synthesizing enzymes, NO precursor and metabolites (citrulline and arginosuccinate) are reported to be localized in different cells (in cerebellum: granule and basket cells for NO synthase and Bergman Golgi epithelial cells for Arg), suggesting a considerable shuttling of NO intermediates. The Arg release may represent its transfer between two cellular compartments, in order to supply NO synthase with its substrate. To investigate the mechanism involved in this shuttling of Arg, release materials from cerebellar slices and cortical astrocytes which were preincubated with [^{3}-H]-Arg were analyzed for labelled and endogenous amino acids. In cerebellar slices, glutamate (500μM), AMPA and kainate (100μM) increased significantly the extracellular level of labelled Arg. Furthermore, it was unaffected by NMDA (100μM) whereas t-ACPD (100μM) gave rise to a delayed Arg increase. Moreover, in astrocyte cultures, an 100% increase in exogenous Arg concentration was also induced by 300μM AMPA. The inhibition of Arg release by excitatory amino acid receptors antagonists is under investigation. These results support the involvement of non-NMDA and metabotropic receptors, probably located on glial cells, in the increase in extracellular level of Arg which is occuring upon selective pathway stimulation both in vitro and in vivo.

S17-12

S-NITROSOGLUTATHIONE IS ENDOGENOUS IN RAT CEREBELLUM.

I. Kluge, U. Gutteck and K.Q. Do. Brain Research Institute, University of Zürich, 8029 Zürich, Switzerland.

The form in which the diffusable and short-lived messenger nitric oxide (NO) is stored, delivered and transported in CNS has not been directly studied. Based on the strong reactivity of NO for thiols and on the presence of cysteine and glutathione at the mM level intracellularly and μM level extracellularly (Zängerle et al., 1992) ,we have investigated whether S-nitrosothiols (RSNO), i.e. S-nitroso cysteine and/or S-nitrosoglutathione (GSNO) may be the potential "package" form in which NO could be stored. We have optimized an extraction method which avoids partial degradation of RSNO and developed a sensitive and selective analytical method based on reversed phase HPLC combined with multiwavelength detection and on-line absorption spectrum which allows to quantify RSNO at a level of 150-300 pmol. In experiments in which cerebellar slices from 8-10 days old rats were incubated with radioactive [^{35}-S]-cysteine and extracted, a radiolabelled peak corresponding to GSNO has been detected. Its identification was confirmed by spiking with reference compound. Indeed, labelled cysteine was taken up and incorporated into glutathione and GSNO. Moreover, the endogenous compound eluting at the retention time of GSNO was chemically characterized by micro HPLC coupled to continous-flow fast atom bombardment mass spectrometry: its fluorenylmethyloxycarbonyl- derivative has a mass spectrum identical to that of authentic GSNO.The use of the same technique and deuterated GSNO as an internal standard will allow us to determine the endogenous concentration of GSNO. The packaging of NO in form of GSNO might serve to facilitate its transport, prolong its life and target its delivery to specific effectors. Furthermore, the formation of GSNO may provide a means to control the toxicity of the free radical NO·.

S17-13

SELECTIVE DISTRIBUTION OF LACTATE DEHYDROGENASE (LDH) ISOENZYMES IN NEURONS AND ASTROCYTES IN THE HUMAN BRAIN P.G. Bittar, Y. Charnay, L. Pellerin*, C. Bouras and P.J. Magistretti*. Département de Psychiatrie, Université de Genève, and *Institut de Physiologie, Université de Lausanne.

In vertebrates, five isoenzymes of LDH are described. At one end of the spectrum, the LDH-5 (muscle type) isoenzyme is thought to favour the formation of lactate from pyruvate, while the LDH-1 (heart) isoenzyme preferentially drives the reaction in the opposite direction. Based on biochemical evidence recently obtained in mouse cerebral cortical cultures (PNAS, 91, 10625-29), we postulated that during activation, astrocytes may be the primary site of glucose uptake and lactate production, while neurons likely utilize lactate as energy substrate. This hypothesis would imply an enrichment of the LDH-1 form in neurons and of the LDH-5 in astrocytes. We report here the production and characterization of two antisera (ABs), 2 and 7, raised in rats immunized with rabbit antigens specific against the LDH-5 and LDH-1 isoforms respectively. Their specificity was verified by immunoblot analysis. Thus, AB2 recognized purified LDH-5 from human placenta and rabbit muscle, both fixed with paraformaldehyde vapors. Pure LDH-1 and rabbit heart extracts were not recognized by AB2. In immunoblots and western blots, AB7 was specific for purified LDH-1 from human erythrocytes and rabbit heart muscle. Immunohistochemistry on 8 post-mortem control human cases revealed a differential cellular distribution of LDH isoforms in the hippocampus : neurons were exclusively stained with AB7 (anti-LDH-1) while astrocytes were stained by both ABs. These observations suggest that neurons process lactate to pyruvate while astrocytes can metabolize glucose glycolytically to produce lactate, as predicted from in vitro studies; the data also support the view of a metabolic exchange between astrocytes and neurons in the human brain.

S17-14

NADPH DIAPHORASE STAINING IN HUMAN PRIMARY VISUAL CORTEX

N. Hadjikhani and S. Clarke; Institut de Physiologie, Université de Lausanne.

Serial coronal sections from two human occipital lobes have been stained for Nissl, myelin, nicotinamide adenine dinucleotide phosphate (NADPH) diaphorase, cytochrome oxydase and acetylcholinesterase. Three types of NADPH diaphorase-positive elements have been found in area 17 and in the underlying white matter. Few stained neurons were found in the cortex mainly in layers II and III; they were non-pyramidal multipolar or, more rarely, bipolar aspiny or sparsely spiny. The white matter beneath the cortex contained a high density of stained neurons; they were either bipolar or multipolar. A rich plexus of labelled axons spread within the cortex and in the underlying white matter. Most axons were thin with boutons en passant. Many of them ran horizontally and some could be followed for up to 500 μm. Occasional basket-like formations were observed in the cortex. Small arterioles, but not capilaries, were surrounded by plexus of fibres rich in boutons.

S17-15

PRIMARY AFFERENT NECK INPUT TO VESTIBULOSPINAL NEURONS, PROJECTING TO THE DORSAL HORN: A DOUBLE LABELING STUDY IN THE RAT AND THE CAT.

S. Bankoul; Institute of Anatomy, University of Fribourg, CH-1700

In rats wheat germ agglutinin-horseradish peroxidase (WGA-HRP) was injected into cervical dorsal root ganglia (DRG; C_2 or C_3) and Fluorogold (FG) into the ipsilateral cervical dorsal horn. WGA-HRP labeled fibers were present, beside the external cuneate nucleus (ECN), in the caudal part of the medial and descending vestibular nuclei (MVN, DVN), while retrogradely labeled FG cells were detectable in the central part of the caudal MVN. A double exposure with fluorescent and polarization optics revealed primary afferent fibers surrounding "basketlike" retrogradely labeled FG cells in the MVN. Similar results were obtained in experiments on cats. After WGA-HRP injections into rostral cervical DRG (C_2-C_5), labeled fibers could be seen, not only in the ECN, but also in the caudal part of the MVN and DVN. In some cases these fibers formed „basketlike" structures, surrounding cells in both nuclei. These findings may represent the morphological basis for a cervico-vestibulo-cervical loop, whose main function could be the maintenance of a certain proportion between the primary afferent information from neck muscles and different secondary afferents, reaching the vestibular nuclei via spinovestibular pathways, and be a kind of a "background noise".

S17-16

THE PARABIGEMINAL NUCLEUS AND ITS TOPOGRAPHIC ORGANIZATION

Robert Kretz and Günter Rager, Universität Fribourg, Anatomisches Institut, rue Gockel 1, CH-1700 Fribourg

The goal of the present study was to investigate the topographic organization of the well developed parabigeminal nucleus in the adult tree shrew (Tupaia belangeri).

Two days following pressure injections of wheat germ agglutinin conjugated to horseradish peroxidase (WGA-HRP) into electrophysiologically defined areas of the dorsal lateral geniculate nucleus (dLGN), the distributions of anterogradely labeled axonal arborizations and of retrogradely labeled cell bodies were studied in coronal sections of the parabigeminal nucleus (PBG). In the nine experiments analyzed so far 3713 labeled neurons have been found in the contralateral PBG. On the contrary, only 81 labeled neurons were counted in the ipsilateral PBG. Additionally, in six of the nine experiments, a strong ipsilateral projection from the dLGN was discovered. The topography of the labeled contralateral neurons and the ipsilateral axonal arborizations are similar. The upper and lower visual fields project to the dorsal and to the ventral parts oft the PBG, respectively. The more peripheral receptive fields are represented in the more lateral part. SNF grant No. 31-36468.92

S17-17

FUNCTIONAL CONSEQUENCES OF VISUAL CALLOSAL CONNECTIONS IN TUPAIA

Robert Kretz, François Mooser and Günter Rager
Universität Fribourg, Anatomisches Institut, rue Gockel 1, CH-1700 Fribourg

Neuroanatomical studies have revealed abundant visual callosal connections in the adult tree shrew (Tupaia belangeri). The goal of the present study was to investigate the functional consequences on neurons of the primary visual cortex.
Single-unit activity from cortical area 17 neurons in the area 17/18 border region was recorded while the callosal influence from the contralateral hemisphere was reversibly inactivated by using the method of cryoblocking. In the five experiments analyzed so far transcallosal interactions could be confirmed on supra- and infragranular neurons. Some neurons showed a decreased activity (up to 90%), some an increased acitivity (up to 35%), and a lot of neurons did not respond to the cryoblocking. The parameters tested were stimulus size, wavelength, intensity, orientation, movement, and direction.
In summary, the above mentioned effects suggest that in the cortical processing of visual information the transcallosal influence might play an important role, even in the adult tree shrew.
Supported by Swiss NSF grant No. 31-36468.92.

S17-18

DIFFERENTIAL DISTRIBUTION OF MAP1A IN THE BARREL CORTEX OF THE ADULT MOUSE.

Touri F., Welker E., Riederer B.M.
Institut d'Anatomie, Université de Lausanne, Rue du Bugnon 9, 1005 Lausanne.

Microtubule-associated proteins (MAPs) are involved in neuronal differentiation. MAP1a, with a apparent molecular weight of 360 KD, is the largest brain MAP and is essential during late maturation of the CNS. In this study, the distribution of MAP1a in the mouse cerebral cortex was investigated with two monoclonal antibodies: A and BW6. The immunostaining of neuronal elements was observed in the barrel cortex, i.e. the part of the somatosensory cortex representing the mystacial vibrissae. Monoclone A stained mainly apical dendrites of pyramidal cells that crossed layer IV between the barrels and having their somata in deeper layers. In contrast, monoclone BW6 stained dendritic processes in the hollow of the barrels. This reveals that in the somatosensory cortex MAP1a is present in (at least) two isoforms, differing in distribution within neuronal elements. One could therefore speculate that MAP1a may be subject to different posttranscriptional modifications in different neuronal populations. Its spatial distribution may therfore be the consequence of regional differences in the cortical processing of sensory inputs. Supported by Swiss NSF grants 31-33447.92 & 31-39184.93.

S17-19

GENETICALLY ENGINEERED POLYMER-ENCAPSULATED CELLS AS A NEW STRATEGY FOR THE TREATMENT OF ALS

N. Déglon*, S.A. Tan*, N. Pochon*, A. Zurn*, E. Baetge#, P. Aebischer*, Division of Surgical Research, Centre Hospitalier Universitaire Vaudois, Lausanne University Medical School; #Cytotherapeutics Inc., Providence, Rhode Island, USA

Amyotrophic lateral sclerosis (ALS) is a progressive and fatal disease characterized by the degeneration of motoneurons. Local delivery of ciliary neurotrophic factor (CNTF) or other neurotrophins (BDNF, NT-3, NT-4/5) have been shown to increase the survival of motoneurons in vitro and in a facial nerve axotomy model in neonatal rats. Before attempting to apply them in humans, the problem of delivery must be resolved. In the present study, we have compared continuous release of CNTF from encapsulated BHK cells with systemic injection of recombinant CNTF protein. Facial nerves of neonatal rats (P2) were transected and CNTF was delivered by one of these methods. 1) direct application of CNTF on the nerve stump using gelfoam (1mg/kg) impregnated with rhCNTF. 2) repeated subcutaneous injections of rhCNTF, 3 times a week. 3) subcutaneous implantation of encapsulated baby hamster kidney (BHK) cells releasing either mCNTF or hCNTF. All three methods of CNTF application significantly improved motoneuron survival with the encapsulated method appearing to be the most effective.
A second aspect that has to be investigated for long-term experiment is the choice of the cell type used for gene delivery. BHK cells divide until they fill the capsule and an accumulation of debris is observed after several months. To overcome this problem, we are currently testing cell lines that can be differentiated into a post-mitotic state. Myoblasts have all the advantages of a rapidly dividing cell line while having the potential of differentiating into post-mitotic myotubes. Mouse C_2C_{12} and rat L6 myoblasts have been transfected with the pNUT expression vector containing the hCNTF gene. The level of expression of the CNTF gene and the bioactivity of the factor were analysed by Northern blot, Elisa assay, and ChAT bioassay. One clone of C_2C_{12} has been found to secrete approximately 0.3-0.5 μg CNTF/10^6 cells/day. Experiments are underway to test the fusion conditions, the effect of long-term encapsulation as well as the *in vivo* viability of these myoblasts as a possible source of neurotrophic factors.

S17-20

TRANSPLANTATION OF GENETICALLY ENGINEERED POLYMER ENCAPSULATED CELLS FOR NEUROLOGICAL DISEASES

P. Aebischer*, A. Zurn*, N. Déglon*, E. Baetge#, , Division of Surgical Research, Centre Hospitalier Universitaire Vaudois, Lausanne University Medical School; #Cytotherapeutics Inc., Providence, Rhode Island, USA

Neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease, or amyotrophic lateral sclerosis (ALS) are characterized by the progressive loss of neuronal elements. This cell loss may be prevented by the localized delivery of neurotrophic factors. This may be achieved by neural grafting of cells genetically engineered to release them. Cell lines offer several advantages, they can be banked, screened prior to transplantation for the presence of pathogens, and efficiently engineered to express and release neurotrophic factors using recombinant DNA technologies. However for potential clinical applications the inherent risk of tumor formation and immune rejection must be minimized. Transplantation of cells isolated within a permselective polymer capsule restricts cell growth to the capsule space and protects them from immune destruction while allowing exchange of molecules between the entrapped cells and host tissue. In the event of capsule breakage, cells from a xenogeneic origin are rejected by the host immune system. For our studies, baby hamster kidney (BHK) cells have been genetically engineered to produce nerve growth factor (NGF), glial cell line derived growth factor (GDNF), ciliary neurotrophic factor (CNTF), brain derived neurotrophic factors (BDNF) and neurotrophin-3 (NT3). The engineered cells and appropriate matrix were loaded within thermoplastic hollow fiber-based capsules. These capsules have been implanted in various models of neurodegenerative diseases such as the pmn/pmn mice model of amyotrophic lateral sclerosis, the transected rat and primate fimbria-fornix model (model bearing some analogy with Alzheimer's disease) and the 6-hydroxy dopamine (6-OHDA) rat parkinsonian model. Significant biological and behavioral improvements were observed in these models with single or multiple combinations of these neurotrophic releasing cells as compared to the capsules loaded with the parent BHK line. A clinical program aimed at the treatment of ALS through the transplantation of encapsulated cells genetically engineered to release CNTF has been initiated.

S17-21

CONSTRUCTION OF A TRANSGENIC MOUSE OVEREXPRESSING HUMAN MONOAMINE OXIDASE B NEURONALY

J. Gottowik, P. Malherbe, A. Cesura, G. Richards, E. Borroni, C. Kühn, H. Blüthmann and M. Da Prada
Pharma CNS, F. Hoffmann-La Roche Ltd, Basel

Transgenic mice which overexpress human monoamine oxidase B (MAO-B) in neurons were generated using the neuron-specific enolase promoter. The F_1-hybrid mice C57 black/6J x DBA2 were used for the genetic manipulation and five lines with different level of expression of the enzyme were obtained. The expressed MAO-B is functionally active. The mice are analysed biochemicaly and histologicaly for neurodegenerative effects. Three different lines are currently interbreed to obtain homozygote mice. A second construction using the GFAP Promoter for overexpressing human MAO-B in astrocytes is in preparation. These transgenic mice could serve as a model for oxidative stress in Parkinson's disease.

S17-22

NERVE GROWTH FACTOR (NGF) MODULATES SINGLE UNIT ACTIVITY IN RAT AUDITORY THALAMUS.

Villa[1] A.E.P., Bajo[2] V., Vantini[1] G., Edeline[3] J.M., de Ribaupierre[1] F. [1]Inst. Physiology, Univ. Lausanne; [2]Univ. Salamanca; [3]URA-1491, CNRS Orsay.

The occurrence of NGF-sensitive cholinergic projections from basal forebrain to discrete regions of auditory thalamus led us to investigate single unit recordings in medial geniculate body and reticular thalamic nucleus after NGF administration. Long-Evans adult rats were icv infused with mini-pumps (Alzet 2002) filled with either 50µg NGF or cytochrome c (controls). After 2-week infusion, single units were recorded in thalamic nuclei under anaesthesia during spontaneous as well as acoustically driven activity. Firing properties were studied with interspike interval histograms and autocorrelograms. Interactions were studied by mean of cross-correlograms. NGF induced a significant increase (50%) of spontaneous firing activity as well as average burst duration. Evoked activity by "white noise" stimulation and the number of responding units were also increased. Half of cell pairs showed a significant correlogram in NGF group whereas only 28% of interactions were significant in controls. Data indicate that NGF ability to affect forebrain cholinergic neurons can be associated with modifications of temporal information processing in auditory thalamus. Sponsored by EEC CHRX-CT93-0269 and Swiss OFES 93.0241 grants.

S17-23

MUTANT P53 AS A PROGNOSTIC MARKER IN ASTROCYTOMAS.

Daub D.M., Tada M., Estreicher A., Iggo R., de Tribolet N. and E.G. Van Meir. Lab of Tumor Biology and Genetics, ISREC, 1066 Epalinges.

We analyzed p53 status in 30 low grade astrocytomas (LGA) with subtotal/total resection of the primary tumor. The presence of mutant versus wild-type p53 was determined using a WTp53-specific transcriptional assay in yeast, and mutations were confirmed by sequencing. The yeast assay showed that mutant p53 was not involved in 5 of the 15 primary tumors which recurred; 4 of these were malignant progressions exhibiting a very short latency period after primary surgery. These represent a group of LGAs where recurrence and/or progression occurs independently of p53 mutation, or where targeting of the p53 pathway occurs by other means. 13 LGAs had a level of p53 mutation exceeding the background level of the assay (set at 10%), and 9 of these recurred (69%); 8 were malignancies with more than 80% mutant p53 present. In contrast, mutant p53 was seen in only 4 of 15 (27%) LGA tumors which have thus far not recurred. These results confirm mutant p53 as an early marker of astrocytic transformation, and suggest mutant p53 can reliably predict malignant evolution and tumor recurrence in a subset of LGA.

S17-24

CLONING OF TUMOR NECROSIS FACTOR-α INDUCED cDNAS IN THE BRAIN

Iris Kemler, Fritz Lahrtz and Adriano Fontana, *Section of Clinical Immunology, Department of Internal Medicine, University Hospital, CH-8044 Zürich*

In infectious diseases of the central nervous system neuronal dysfunction is common, such as disturbance of consciousness or epileptic seizures. One of the cytokines released in the brain in viral infections is tumor necrosis factor-α (TNFα). In order to understand the interaction of TNFα with neurons we have set up a system allowing the identification of cDNAs which are induced by this cytokine. A cDNA library of TNFα treated neuroblastoma cells (Neuro2a) was screened with a probe enriched for TNFα induced mRNAs. One of the genes isolated so far is IκB, the inhibitor of the transcription factor NFκB. IκB transcripts are induced 5-fold by TNFα in Neuro2a cells and to a lesser extent also in mouse cerebellar neurons. Currently the different members of the NFκB/Rel and IκB families are being characterized in the mouse brain. Furthermore, we are screening for other TNFα induced genes.

S17-25

CLONING AND CHARACTERISATION OF KYNURENINE AMINOTRANSFERASE FROM RAT BRAIN.

D. Alberati-Giani, P. Malherbe and A.M. Cesura.
Pharma Division, Preclinical Res., F. Hoffmann-La Roche Ltd, CH-4002 Basel, Switzerland.

In this work, we report on the molecular identity of rat brain kynurenine aminotransferase (KAT). Amino acid sequencing of rat kidney KAT triptic peptides revealed that KAT and glutamine transaminase K (GTK) represent the same molecular entity. After RT-PCR of rat brain mRNA using primers derived from the cDNA described for GTK, the oligonucleotide sequence of the isolated PCR fragment was found to be identical to that reported for GTK. HEK-293 cells tranfected with the KAT cDNA clone isolated from rat brain showed KAT activity with kinetic properties identical to those of native KAT from rat brain and kidney.

S17-26

AGONIST INDUCED DOWN-REGULATION OF NMDA RECEPTORS IS MIMICKED BY INHIBITION OF Ca^{2+}/CaM KINASES

M.Villa, A.Resink*, D.Benke, H.Möhler and R.Balázs*
Inst.of Pharmacology, ETH and University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland,
* Netherlands Institute for Brain Research, Amsterdam

NMDA receptors play a critical role in synaptic plasticity and neuro-toxicity. NMDA treatment of cerebellar granule cell cultures evoked a down-regulation in NMDA receptor activity, as assessed by NMDA-induced $^{45}Ca^{2+}$ influx. A corresponding reduction in the protein levels was detected by Western blotting (NR1 subunit antiserum) and photoaffinity labeling (specific ligand ^{125}I-CGP55802A).In contrast, NR1 mRNA levels recovered to control levels despite the continuous presence of the agonist. The receptor down-regulation was reversible, since both NMDA-induced $^{45}Ca^{2+}$ influx and protein levels were restored to control levels upon NMDA removal. To study the mechanism involved, protein phosphorylation was investigated. While PKC-inhibitors were unable to influence the down-regulation process, KN62, a specific inhibitor of Ca^{2+}/CaM kinases II and IV, elicited a down-regulation of NMDA receptors even in the absence of NMDA. Thus, post-transcriptional events seem to play a critical role in NMDA receptor down-regulation. In particular, the process can be mimicked by inhibition of Ca^{2+}/Calmodulin-dependent kinases II and IV.

S17-27

Search for cDNAs expressed in the mouse substantia nigra.

A. Savioz* and R. W. Davies, Robertson Lab. of Biotechnology, Uni Glasgow.

The genetic and molecular basis of differentiated neuronal phenotype is essentially unknown. Central roles must be played by genes with restricted, specific expression patterns, while some characteristics of cell physiology will depend upon shifts in the relative expression of less specific genes. We are particularly interested in genes that determine the phenotypes of mammalian dopaminergic neurons in the ventral midbrain. We set out to generate a set of cDNA clones that are expressed in dopaminergic neurons, in order to contribute to their description at the molecular level. Within this class of genes there must be a small subset which contribute a large proportion of the genetic variation in susceptibility to Parkinson's disease. A reasonable working hypothesis is that such genes are more likely to have non-uniform patterns of gene-expression such as may be detected by screening for differential expression between brain regions.
Here we present, as a first level of analysis, data from a differential screen of a sample of clones from an unsubtracted ventral midbrain library, with and without presceening to reduce the number of cDNAs corresponding to exceedingly common mRNAs, *in situ* hybridizations and differential fragment blot, and, as a second level, the production and analysis of mouse ventral midbrain subtracted libraries.

S17-28

THE RAT HOMEOBOX GENE PBX1 IS CONTINUOUSLY EXPRESSED IN NEURONS MIGRATING FROM THE SUBVENTRICULAR ZONE TO THE OLFACTORY BULB.

Lori Redmond*, Susan Hockfield* and Maria A. Morabito[+]
*Section of Neurobiology and [+]Department of Pharmacology, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06520-8066, USA.

Within the mammalian telencephalon, an important phase of neurogenesis occurs postnatally in the subventricular zone. Cells generated in this zone continue to divide and undergo cell death as they migrate to the olfactory bulb, where they differentiate into interneurons. Previous findings in the hematopoietic system indicate that the human homeobox gene PBX1, which codes for a putative transcriptional repressor, is involved in the choice of proliferation versus differentiation and apoptosis.

To investigate the role of PBX1 in mammalian neurogenesis, we cloned and characterized the expression of the rat homologue of human PBX1. In the central nervous system, PBX1 is expressed postnatally at high levels in the anterior part of the subventricular zone, the site where olfactory interneurons are generated. PBX1 expression is maintained by these unique neurons through several stages of their differentiation and migration into the olfactory bulb.

The sustained expression of rat PBX1 in these regions strongly suggests that, in the nervous system as in the hematopoietic system, PBX1 plays an important role in the regulation of eukaryotic cellular events such as proliferation, differentiation and apoptosis.

Molecular biology of parasites

S18-01

REGULATED SECRETION IN TOXOPLASMA GONDII: WHAT TARGETS ROP1 TO THE RHOPTRIES? (D. Soldati[3], J. Kampmeier[1], J.F. Dubremetz[2], J. C. Boothroyd[1]). [1]Dept Microbiol. & Immunol. Stanford University School of Medicine, Stanford CA 94305. [2]Unité 42 INSERM 596511 Villeneuve d'Ascq FRANCE. Zentrum für Molekulare Biologie, Universität Heidelberg).

Toxoplasma gondii is an obligate intracellular protozoan parasite that is capable of infecting virtually all types of cells.
The cellular structure of the parasite is of particular interest with respect to invasion. As a member of the Apicomplexa, this organism possesses several specialized organelles called micronemes, rhoptries and dense granules. Secretion by these organelles is regulated and is presumed to be an important sequential event in the establishment of intracellular parasitism. The signals targeting specific proteins to their respective secretory organelles are not known. In addition, all the micronemes and rhoptry proteins studied so far are post-translationally processed during their transport to the appropriate organelles but the purpose of the processing is unknown.
A knock out mutant of the rhoptry protein gene *ROP1* has been obtained using chloramphenicol as drug selection. Such parasites are viable indicating that *ROP1* is not an essential gene. The ROP1 knock-out strain has been used to study the signal directing ROP1 to the rhoptries. From this strain, recombinant parasites stably expressing truncated forms of ROP1 or chimeric molecules of ROP1 and GRA3 (a dense granule protein) were selected using phleomycin. Immunofluorescence and immuno-electronmicroscopy using anti-ROP1 antibodies were used to detect and determine the cellular localization of the engineered ROP1 in the recombinants. In addition we have investigated what is the default secretory pathway in Toxoplasma by fusing the N-terminal secretory signal peptide (from the toxoplasma major surface antigen SAG1) to normally cytosolic proteins.

S18-02

DIFFERENTIAL *IN VITRO* MAINTENANCE OF *ECHINOCOCCUS MULTILOCULARIS* METACESTODE. Gottstein Bruno & Andrew Hemphill, Institute of Parasitology, University of Berne.

Infection with the larval stage (metacestode) of *Echinococcus multilocularis* causes alveolar echinococcosis predominantly in rodents and accidentally also in humans. Human patients and murine *in vivo* models have shown that the immunogenetic background of a host can predetermine growth rate and fertility of the metacestode. In order to assess parasite-specific regulation of metacestode differentiation and respective regulation of gene expression independent of the complex host influence, we developed host-independent *in vitro* models for cultivating *E. multilocularis* metacestode. We documented growth of non-fertile metacestode vesicles surrounded by the typical laminated layer, and, under certain and defined conditions, protoscolex formation *in vitro*. Fertile and non-fertile *in vitro* grown vesicles were comparatively assessed with respect to their antigenic profiles, their morphological substructures (with special emphasis on EM and SEM analyses of the laminated layer) and their proliferative growth potential. Our models allow to study for the first time host-independent parasite biology, including stage-specific gene-expression, cell differentiation and energy and drug metabolism.

S18-03

SIMULTANEOUS IDENTIFICATION OF *NEOSPORA CANINUM* AND *TOXOPLASMA GONDII* BY DUAL AMPLIFICATION OF SPECIES-SPECIFIC GENES IN POLYMERASE CHAIN REACTION (PCR)

Yamage, M., Kaufmann, J., Gottstein, B. Institute of Parasitology, University of Berne, Berne.

The newly identified apicomplexan parasite *Neospora caninum* and the feline coccidia *Toxoplasma gondii* are morphologically similar at their motile tachyzoite stages. With a lack of information on the complete *Neospora* life-cycle, a reliable identification method is needed. We developed a *Neospora caninum*-specific DNA probe and respective oligonucleotides which can be used as primers for amplifying *Neospora caninum*-specific DNA fragments in the polymerase chain reaction (PCR) for differential diagnosis.

Seven 19-mer oligonucleotide primers (sense and antisense) were synthesized based on the sequence of the *Neospora caninum*-specific DNA probe. Using four sets of primers, single DNA fragments of predicted sizes were amplified with the genomic DNA as a template in a highly species-specific fashion. In combination with B1 gene-based primers specific for *Toxoplasma gondii*, both *Neospora caninum* and *Toxoplasma gondii* DNAs were clearly discriminated by the specific DNA fragments amplified in a single reaction. Unlike the antibody or antigen detection, which might vary depending upon the developmental life-cycle stages, the DNA based diagnosis could provide unequivocal etiological evidence of *Neospora caninum*.

S18-04

ADHESION AND INVASION OF ENDOTHELIAL CELLS BY *NEOSPORA CANINUM* TACHYZOITES
Hemphill, A., Kaufmann H., and Gottstein B., Institut für Parasitologie, CH-3012 Bern

***Neospora caninum* is a recently identified coccidian parasite which was, until 1988, midiagnosed as *Toxoplasma gondii*. It causes paralysis and death in dogs and neonatal mortality and abortion in cattle, sheep, goats and horses. The life cycle of *Neospora* has not yet been elucidated. The only two stages identified so far are tissue cysts and intracellularly dividing tachyzoites. Very little is known about the biology of this species.**
We have set up a fluorescence-based adhesion/invasion assay in order to investigate the interaction of *Neospora caninum* tachyzoites with bovine aorta endothelial (BAE) cells in vitro. Treatment of both host and parasites with metabolic inhibitors determined the metabolic requirements for adhesion and invasion. Chemical and enzymatic modifications of parasite and endothelial cell surface was used in order to obtain information on the nature of cell surface components responsible for the interaction between parasite and host. Electron microscopical investigations defined the ultrastructural characteristics of the adhesion and invasion process, and provided information on the intracellular development of the parasites.

S18-05

IMMUNOLOGICAL RESPONSE IN MICE TO A RECOMBINANT *GIARDIA* VSP-ANTIGEN, EXPRESSED IN A *S.ALMONELLA* VACCINE
Müller, N., Stäger, S., Zimmermann, V. and Gottstein, B., Institut für Parasitologie, CH-3012 Bern

Giardia lamblia is a protozoan parasite which resides in the small intestine of humans and various animal species. The disease is characterized by a considerable variation in the development of the infection. Manifestations of *G. lamblia* infections vary from asymptomatic carriage to severe diarrhea and maladsorption. A few years ago, we have initiated a project focused on the immunobiology of the parasite. Experimental *G. lamblia* infections of mice revealed that the course of the infection is dependent on the immunological status of the animals. In these experiments we could demonstrate that *G. lamblia* specific T- and B-cell functions are essential both for the induction of self-healing and for the development of immunity. Additionally, our studies indicated a significant correlation between the production of intestinal IgA directed against the parasites' major surface antigen VSP (variant specific surface protein) and an antigenic diversification of the parasite population in the gut. In order to investigate the cellular mechanisms of the VSP specific immune response, we have recently chosen a molecular biological approach which allowed us to perform an intestinal immunization of mice with VSP as sole component of the parasite. This could be achieved by producing VSP in an attenuated *Salmonella typhimurium* strain and by subsequently using this recombinant strain as a peroral live vaccine. Lymphocyte proliferation assays revealed that immunization with recombinant *Salmonella* induces a cellular intestinal immune response specifically directed against VSP. On the basis of these results, further experiments will be designed which shall explore the immunological functions of VSP in*G. lamblia* infected mice.

S18-06

COMPARATIVE GENETIC ANALYSIS OF SWISS AND SPANISH ISOLATES OF *ECHINOCOCCUS GRANULOSUS*
M. Siles, R. Felleisen, & B. Gottstein; Institute of Parasitology, University Bern, Switzerland

For a comparative genetic analysis, Swiss and Spanish isolates of *E.granulosus* were characterized using two molecular biological techniques: Genomic DNAs isolated from parasites originating from various intermediate hosts were subjected to Southern hybridization with different probes, the same DNA was used for DNA amplification using the Random Amplified Polymorphic DNA (RAPD) technique. With both methods the various isolates of *E. granulosus* exhibited characteristic banding patterns which allowed us to assign them to the following groups of homologous profiles: (a) isolates of horse/donkey origin from Spain and Switzerland; (b) isolates of cattle origin from Switzerland; (c) isolates of sheep, cattle and human origin from Spain; (d) isolates of pig origin from Spain and Switzerland and of goat origin from Spain. The results provide further evidence that the morphological and biological differences of several strains of *E. granulosus* are also detectable on the genetic level using molecular biological methods and demonstrate the usefulness of the RAPD technique for subspeciation purposes in parasites.

S18-07

AN ALTERNATIVE APPROACH FOR THE CONSTRUCTION OF PARASITE cDNA LIBRARIES FROM LOW AMOUNTS OF RNA

R. Felleisen & B. Gottstein; Institute of Parasitology, University Bern, Bern, Switzerland

Stage specific cDNA libraries from the small fox tapeworm *Echinococcus multilocularis* could represent a valuable tool for the characterization of stage-specific gene expression and identification of the respective antigens, which can serve for different applications e.g. for an analysis of their corresponding biological function, as diagnostic antigens or potential vaccine candidates. However, the isolation of parasite material in amounts sufficient for RNA purification and subsequent cDNA library construction by classical molecular biological methods is very difficult in cases where the parasite stage of interest cannot be cultivated *in vitro* and/or be purified in large amounts from natural sources i.e. especially the egg and oncosphere stages. We therefore developed a novel technique based on cDNA amplification by PCR using arbitrary primers which allows the construction of cDNA libraries with minute amounts of total RNA as starting material. Using this technique cDNA libraries from different stages of *E.multilocularis* have been constructed and are presently screened for T-cell epitope expression.

S18-08

SYNTHESIS AND IMMUNOLOGICAL CHARACTERIZATION OF 104-MER AND 102-MER PEPTIDES CORRESPONDING TO THE N- AND C-TERMINAL REGIONS OF THE *PLASMODIUM FALCIPARUM* CS PROTEIN

Roggero M.A., Filippi B., Church P., Hoffman S.L., Blum-Tirouvanziam U., Esposito F., Matile H., Reymond C.D., Fasel N. and Corradin G.
Institute of Biochemistry, University of Lausanne.

We investigated the immunogenicity and the conformational properties of the non-repetitive sequences of the *P. falciparum* circumsporozoite (CS) protein. Two polypeptides of 104 and 102 amino acids long, covering respectively the N- and C-terminal regions of the CS protein were synthesized using solid phase Fmoc chemistry. Sera of mice immunized with the polypeptides in adjuvant reacted with the synthetic polypeptides as well as with native CS protein. Sera derived from donors living in a malaria endemic area recognized the CS 104- and 102-mers. Conformational studies of the CS polypeptides were also performed by circular dichroism spectroscopy showing the presence of a weakly ordered structure that can be increased by addition of trifluoroethanol. The obtained results indicate that the synthetic CS polypeptides and the natural CS protein share some common antigenic determinants and probably have similar conformation.

RNA processing and translation

S19-01

CLONING OF cDNAs ENCODING MAMMALIAN dsRNA-SPECIFIC ADENOSINE DEAMINASE.

Sabine Krause, Mary A. O'Connell and Walter Keller, Biozentrum, University of Basel, CH-4056 Basel.

The double-stranded (ds) RNA-specific adenosine deaminase, which converts adenosine to inosine, uses dsRNA as substrate. This enzyme is probably involved in the mRNA editing of the GluRB subunit of the glutamate-gated ion-channels in the brain, as in this editing event a specific adenosine is converted to inosine (Dr. P. Seeburg, Nature, submitted). Partially purified adenosine deaminase also contains GluRB pre-mRNA editing activity.

Tryptic peptide sequences were obtained from the purified bovine dsRNA adenosine deaminase (116 kD). Specific DNA probes were constructed by PCR and a partial bovine cDNA clone was isolated. This clone was then used to probe HeLa and rat libraries to obtain full length cDNA clones.

A histidine affinity tag was fused to the partial bovine cDNA clone, and antibody raised against it. This antibody depleted an extract of deaminase activity, which was restored by addition of pure protein, proving that we have purified and cloned the protein responsible for the dsRNA deaminase activity.

We are currently attempting the purification of additional components required for GluRB pre-mRNA editing.

S19-02

Characterization of mRNA 3'-end formation in yeast

Pascal Preker, Joachim Lingner, Lionel Minvielle-Sebastia, and Walter Keller. Abt. Zellbiologie, Biozentrum der Uni Basel.

In yeast, as in higher eukaryotes, the mature 3'-end of most mRNAs is generated by cleavage of the primary transcript followed by polyadenylation of the 5'-cleavage product. So far, poly(A) polymerase (*PAP1*) is the only enzyme that has been purified to homogeneity and cloned. In an attempt to find additional factors involved in 3'-end formation, we have applied two strategies that identify genes on the basis of their genetical or physical interaction with *PAP1*.

(i) Two previously described temperature-sensitive mutants, *rna14* and *rna15*, were found to be synthetically lethal with conditional mutants in *PAP1*. Protein extracts from these mutants are deficient for both cleavage and polyadenylation of mRNA precursors. Processing activity can be complemented by the addition of partially purified Cleavage Factor I (CFI), suggesting that *RNA14* and *RNA15* encode subunits of CFI.

(ii) A two-hybrid screen for proteins that interact with PAP1 identified a novel gene termed *FIP1* (*f*or*f*actor *i*nteracting with *P*AP1). Protein extracts from conditional mutants in FIP1 specifically cleave mRNA precursors *in vitro*, but fail to polyadenylate the 5'-cleavage product. Polyadenylation activity can be restored by the addition of partially purified fractions containing Polyadenylation Factor I (PFI).

S19-03

A NOVEL APPROACH TO SCREEN FOR RNA-BINDING PROTEINS OF SIMILAR SPECIFICITY

Henderson,B., Menotti,E., Kühn,L., ISREC,Epalinges

Two iron regulatory proteins (IRP and IRP_B) control post-transcriptionally the use of several mRNAs that encode proteins involved in iron metabolism. IRP and IRP_B bind in vitro with similar affinity to RNA stem-loop structures called iron responsive-elements (IREs). The 6 nucleotides forming the IRE loop (CAGUGN) and the bulge (cytosine) are conserved. Previously, in vitro selection from a randomized IRE pool predicted base-pairing within the IRE loop between position 1 and 5. We now describe a new approach employing pools of IRE-like RNAs randomized at different loop positions to search for new RNA-binding proteins. We fractionated cytoplasmic extract from B16 melanoma cells on a Mono-Q column. With the wild type IRE, we could identify IRP and IRP_B containing fractions. However, neither with this probe nor randomized IRE probes of different degeneracy could we identify additional sequence-specific RNA-binding proteins. Finally we demonstrate that the 1-5 base-pairing within the IRE loop is important for binding of both IRP and IRP_B.

S19-04

ISOLATION OF THE TRANSLATION INITIATION FACTOR PRT1 FROM *SACCHAROMYCES CEREVISIAE*

P.Danaie, B.Wittmer, M.Altmann and H.Trachsel
Institute of Biochemistry and Molecular Biology, University of Berne, 3012 Berne, Switzerland

Translation initiation factor Prt1 was purified from a ribosomal salt wash fraction of *Saccharomyces cerevisiae* cells. Prt1 protein cofractionates with four other polypeptides during all steps of purification suggesting that it is part of a protein complex containing polypeptide subunits with apparent molecular weights of 130, 80, 75 (Prt1), 40 and 32kD. The Prt1-containing protein complex has RNA-binding activity and is an active translation factor as shown by its ability to restore translation in a cell-free system derived from a temperature-sensitive prt1 mutant strain in which endogenous Prt1 activity is inactivated by heating the extract to 37°C.

S19-05

STAGE SPECIFIC & ANTI-SENSE TRANSCRIPTION IN THE HISTONE H1-LIKE LOCUS OF *LEISHMANIA MAJOR*

Tanja Noll, Chantal Desponds, Theresa Glaser and Nicolas Fasel, Institut de Biochimie, UNIL.

Leishmania is a protozoan pathogen responsible for a wide range of human diseases. *L.major* displays a digenetic life cycle characterized by an extracellular promastigote in the insect vector, and an intracellular amastigote in the host macrophage. *In vitro* grown promastigotes undergo sequential differentiation from a non-infective to an infective form associated with a change from logarithmic to stationary phase of growth. Using differential cDNA hybridization techniques, we isolated the sw3 gene which shows greater expression at the RNA level in the amastigote form. Primary results indicate that sw3 may be differentially regulated during promastigote maturation. The sw3 gene encodes an histone or H1-like protein. Structural analysis of this gene and its neighbouring sequences shows that additional polypeptides could be encoded by the opposite strand of this DNA segment. *In vitro* translation confirmed the presence of ORFs on both strands. Sense and anti-sense transcripts were detected *in vivo* suggesting transcription in both directions.

S19-06

CHARACTERISATION OF THE HAIRPIN BINDING FACTOR INVOLVED IN HISTONE PRE-mRNA PROCESSING

A. Schaller and B. Müller

Zoologisches Institut, Abteilung für Entwicklungsbiologie, Universität Bern, Baltzerstrasse 4, 3012 Bern

The mature 3' ends of replication-dependent histone mRNAs are formed by an endonucleolytic cleavage reaction. Important cis-acting sequences are a hairpin structure 5' of the cleavage site and a spacer element 3' of the cleavage site. Using mouse cell nuclear extracts competent in this processing reaction, we have characterised one of the trans-acting factors involved. We demonstate that the binding of the so-called hairpin binding factor (HBF) to histone (pre-)mRNA is structure-, as well as sequence-specific and that the resulting complex is extremely stable *in vitro*. Preliminary evidence suggests that HBF is a multiprotein complex and that its RNA-binding component has an apparent molecular weight of 45 kDa.
We are currently purifying the HBF and progress in the purification will be presented.

S19-07

INTERACTIONS BETWEEN HUMAN SPLICING FACTORS SF3a, SF3b AND U2 snRNP : A STRUCTURAL MODEL

P. Grüter, D. Nesic, C. Wersig, K. Gröning and A. Krämer
Département de Biologie Cellulaire, Université de Genève, 30, quai Ernest-Ansermet, 1211 Genève 4

Pre-mRNA splicing is catalyzed by a multicomponent complex, the spliceosome, that is assembled in a series of dynamic interactions between pre-mRNA, snRNPs and 30 to 50 protein factors. Splicing factors SF3a and SF3b are part of 17S U2 snRNP that is essential for the assembly of pre-splicing complex A. SF3a consists of three tightly associated polypeptides of 60, 66 and 120 kD. cDNAs encoding these proteins have been isolated and regions in $SF3a^{60}$ and $SF3a^{120}$ that are essential for an interaction between the subunits have been defined. Recombinant wild-type and mutant polypeptides will be used to reconstitute the SF3a complex in order to map additional functional domains in in-vitro assays. SF3b consists of at least four polypeptides two of which bind directly to U2 snRNA. Immunoprecipitation experiments demonstrate that contacts of SF3a with both SF3b and the U2 snRNP are required during the formation of the active 17S U2 snRNP; these interactions persist in the assembled particle. Protein-protein crosslinking will provide more insight into these interactions.

S19-08

MOLECULAR ANALYSIS OF THE STRUCTURE AND FUNCTION OF MAMMALIAN SPLICING FACTOR SF1

S. Backes, R. Gatto, X. Yang and A. Krämer
Département de Biologie Cellulaire, Université de Genève, 30, quai Ernest-Ansermet, 1211 Genève 4

Introns are spliced from nuclear pre-mRNA in multicomponent complexes that are assembled in a stepwise fashion by the combined action of snRNPs and non-snRNP protein factors. Interactions of U1 snRNP, SR-proteins and U2AF commit the pre-mRNA to the splicing pathway. Subsequently the 17S U2 snRNP binds to the intron generating presplicing complex A, a reaction that also requires SF1, a heat-stable protein of 75 kD. cDNA clones encoding putative alternatively spliced variants of SF1 have been isolated and at least two mRNAs are differentially expressed in various cell lines and tissues. Recombinant SF1 expressed in insect cells functions in presplicing complex formation in vitro. SF1 is composed of several structural domains: a putative leucine-zipper, a zinc-knuckle and a proline/glutamine-rich C-terminal half. All of these regions may contact other components of the splicing apparatus, e.g. proteins, snRNPs or the pre-mRNA. In addition, our own data as well as sequences retrieved from current data bases demonstrate the presence of at least four isoforms of SF1 with divergent C-terminal domains. We are currently investigating the function of the individual domains by mutational analyses. A Drosophila cDNA library has been screened for a SF1 homologue to provide a system for genetic and developmental studies.

Cell cycle and apoptosis

S20-01

Coincidence Analysis of Genes expressed in different Systems undergoing programmed Cell Death

Guo Ke, Zhiwei Feng, Susanne Bührer, Susanne Saurer and Robert Friis

Departement Klinische Forschung, Universität Bern, Tiefenaustrasse 120, CH-3004 Bern

A Coincidence Analysis led to the isolation of five genes from involuting rodent mammary and prostate glands. Two of these genes had been previously identified in other contexts. ***IAP*** has been reported to be an $\alpha_v\beta_3$ integrin-associated protein with Ca^{++} regulator function (Schwartz et al., J. Biol. Chem. 268: 19931; 1993) and ***Gas-1*** has been previously described as a growth arrest gene for NIH 3T3 cells (Del Sal et al., Cell 70: 595; 1992), dependent on ***p53*** for function. We have established that both ***IAP*** and ***gas-1*** expression occur in cells entering apoptosis in response to physiological hormone-dependent signals in prostate, mammary gland, uterus and ovary. Over-expression studies are in progress, in which anlage-denuded mammary fat pads are re-implanted with normal mammary epithelial cells infected with retroviral, ***IAP*** - or ***gas-1***-expressing constructs.

S20-02

IDENTIFICATION OF PROTEINS WHICH INTERACT WITH THE SURVIVAL FACTOR BCL-2

Otter, I., and Borner, C. Institute of Biochemistry, University of Fribourg, 1700 Fribourg

The oncogene product Bcl-2 efficiently protects mammalian cells from programmed cell death induced by a variety of toxic and physiological agents. How Bcl-2 achieves this extraordinary effect is still an enigma. One approach to shed light on the underlying molecular mechanisms is to identify proteins which interact with Bcl-2 and may therefore serve as regulators or target substrates of the survival protein. Recently Bcl-2 binding proteins have been reported. Among them are Bcl-2 homologs such as Bax and Bcl-x which function mainly as positive or negative regulators of Bcl-2. We attempted to isolate more binding proteins by two strategies: Passing a cellular extract through an affinity column containing a glutathione S-transferase (GST)-Bcl-2 fusion protein, and co-immunoprecipitating binding proteins with a Bcl-2 antibody from metabolically labeled cellular extracts. The latter method has the additional advantage to identify binding proteins which interact with Bcl-2 only in response to cellular stresses. We present preliminary data on the identification of Bcl-2 binding proteins by both isolation strategies. Purification and molecular characterization of the proteins are in progress.

S20-03

IMPACT OF THE SURVIVAL FACTOR BCL-2 ON PROTEIN TURNOVER

Favre, D. and Borner, C., Institute of Biochemistry, University of Fribourg, Rue du Musée 5, CH-1700 Fribourg

The proto-oncogene bcl-2 is implicated as a component of the molecular processes that decides whether a cell lives or dies. We analyzed the impact of bcl-2 protein expression on host cell protein synthesis and stabilization. Pulse-chase experiments revealed that bcl-2 expression resulted in enhanced degradation of polypeptides after translation. Pulse labeling revealed that bcl-2-expressing cells showed a complex modulation of protein synthesis as compared to control cells. These observations were strengthened in cells which were stressed with various stimuli.

The impact of bcl-2 expression on host cell protein synthesis and degradation of host polypeptides by the ubiquitin degradation pathway will be discussed.

S20-04

BCL-2 SLOWS DOWN THE PROLIFERATION OF EUKARYOTIC AND PROKARYOTIC CELLS

Borner, C., Institute of Biochemistry, University of Fribourg, Rue du Musée 5, 1700 Fribourg

There is emerging evidence that mammalian cells are most susceptible to programmed cell death (apoptosis) when they are in a extensively proliferating, high-turnover state. The oncogene product Bcl-2 protects cells from apoptosis, but the mode of its action is still unknown. An attractive possibility would be that Bcl-2 confers death resistance by decreasing the rate of cell turnover. Indeed, I found that not only several mammalian cell lines, but also yeast and bacteria exhibited slower growth rates when overexpressing Bcl-2. This was a direct consequence of the action of Bcl-2 because it depended on the level of Bcl-2 expression and could be achieved in a cellular system where Bcl-2 expression was inducible. These results point towards a so far unanticipated, negative impact of Bcl-2 on cell proliferation.

S20-05

THE INTERACTION OF BCL-2 WITH THE RAF-SIGNALLING PATHWAY

Olivier, R., and Borner, C. Institute of Biochemistry, University of Fribourg, Rue du Musée 5, 1700 Fribourg

It has recently been reported that the survival factor Bcl-2 interacts with the Ras-related protein R-ras. R-ras in turn was shown to associate with the proto-oncogene product Raf. Raf itself can protect cells from programmed cell death (apoptosis) and appears to directly associate with the survival factor Bcl-2 in a baculovirus expression system. These data suggested that Bcl-2 may use a R-ras-Raf signal transduction pathway to exert its survival function. If this were true, expression of a dominant-negative Raf mutant would ablate the survival activity of Bcl-2. Here we show that fibroblasts overexpressing such a Raf mutant in addition to Bcl-2 are however still protected from apoptosis induced by brefeldin A, okadaic acid and staurosporine. This indicates that Bcl-2 may use a Raf-independent signalling pathway to fulfill its death-protective function.

S20-06

DETERMINATION OF N-TERMINAL PHOSPHORYLATION REQUIRED FOR MITOTIC ACTIVATION OF SRC TYROSINE KINASE

Meili, R and Ballmer-Hofer, K
Friedrich Miescher Institute, Basel, Switzerland

The tyrosine kinase Src is activated in mitosis. At the same time it is phosphorylated in the N-terminal "unique" domain at the three amino acids T34, T46, S72. The same sites can be phosphorylated in vitro by cdc2/Cyclin B kinase. Previous experiments have shown that some or all of these phosphorylations are required for mitotic activation in vivo.
To determine the precise phosphorylation requirements for activation we mutated each phosphorylation site to alanine.
We combined these mutations in all possible permutations and tested which of the resulting proteins were still activated during mitosis.

S20-07

Functional studies with a CDK2-variant from hamster

Ellenrieder C. and Jaussi R., Institut für Medizinische Radiobiologie, Universität Zürich & Paul Scherrer Institut, CH-5232 Villigen-PSI

In mammalian cells CDK2 (p33, cyclin-dependent kinase) is involved in cell cycle regulation. Its activity is regulated by interaction with cyclins and other regulatory proteins as well as by phosphorylation. After DNA damage induced by drugs or radiation, it is hyperphosphorylated and the cell cycle is arrested. The release of this arrest by caffeine induces a dephosphorylation of CDK2. A novel p40 CDK2 variant, which displays a similar phosphorylation pattern after the same treatments has been detected on Western blots with anti-PSTAIRE-antibodies (Hain et al., Cell. Signal., 6(5), 1994). Up to date, this protein has been detected in Syrian and Chinese hamster as well as in mouse but not in man. Hamster and mouse cells typically display a relaxed mitotic control as opposed to human cells with a stringent mitotic control (Schimke et al., CSH Symp. Quant. Biol., LVI, 1991). With the exception of an insertion of 144 bp in the long variant, the two CDK2 forms of Syrian hamster share the same mRNA sequence (Noguchi et al., BBRC, 197(3), 1993). The insert sequence is identical in Chinese and Syrian hamster. We suggest that p40 might contribute to relaxation of the mitotic control. We are now constructing a human-hamster hybrid cDNA encoding a humanised p40 and will express it in human HeLaS3 cells with the hope to provoke a relaxation of the mitotic control in HelaS3 cells and/or a change in the cells' radiosensitivity.

S20-08

QUANTIFICATION OF RAPID PHOSPHORYLATION OF CELL CYCLE KINASES INDUCED BY IONIZING RADIATION

Forrer P. and Jaussi, R., Institut für Medizinische Radiobiologie, Paul Scherrer Institut & Universität Zürich, CH-5232 Villigen

In the presence of unreplicated or damaged DNA entry into mitosis is delayed or blocked in most eukaryotic cells. The ability of cells to arrest at the G2/M border seems to be most important for cell survival after irradiation. This is the time point when transformed cell lines mainly arrest the cell cycle after irradiation by rapid phosphorylation of CDK1. *In vivo*, this arrest leads to an enhanced survival of irradiated tumor cells partly due to extended repair time. To better understand the involved signaling pathways we are developing an indirect ELISA test. The wells of a microtiter plate are coated with purified cell cycle kinases, blocked, and cell extracts from irradiated cells are applied. After washing, the amount of phosphorylation of each of the kinases is quantified by using antibodies against phosphoaminoacids. This method allows kinetic analysis of the pathway by monitoring its interaction with the key proteins of the cell cycle machinery. The effects of chemicals (e.g. caffeine) on this signal transduction can be examined as well.

S20-09

CLONING AND CHARACTERISATION OF GENES REGULATING CYTOKINESIS IN *S.POMBE*.

C.Fankhauser, S.Schmidt, L.Cerutti and V.Simanis, ISREC, 1066 Epalinges, Switzerland

Little is known about the mechanisms which regulate cytokinesis and how it is integrated with mitosis. We study a number of genes which seem to play a role in coordinating mitosis with cytokinesis. Defects in the *cdc7, cdc11* and *cdc14* genes inhibit septum formation but the cells continue to grow and undergo nuclear divisions, leading to highly elongated multinucleate cells. Genetic analysis suggests that these gene products may interact. We have cloned the genes *cdc7* and *cdc14* by complementation. *Cdc7* encodes a protein kinase which is a dosage dependent regulator of septum formation, and the *cdc14* gene product is required for septum formation and its overexpression inhibits entry into mitosis.

Cdc11 plays a key role in the regulation of septum formation. Attempts to clone *cdc11* by complementation produced only multicopy suppressors, sup11-3 and sup11-6. Characterisation of these two suppressor genes will be presented. We are currently cloning the *cdc11* gene by two means: chromososme walking and the Two-Hybrid system.

S20-10

TEMPORAL AND SPATIAL CONTROL OF CYTOKINESIS IN THE FISSION YEAST *S. POMBE*

M. Sohrmann, S. Utzig, L. Cerutti, C. Brodbeck, C. Fankhauser, and V. Simanis, ISREC, 1066 Epalinges, Switzerland

We are studying the regulation of the onset of cytokinesis and its coordination with mitosis. The earliest morphological event after the onset of mitosis is relocation of actin from the growing tips of the cell to form a medial actin ring whose position anticipates that of the division septum. Its usual location is at the middle of the cell, at the position of the interphase nucleus. Our present work is focused upon two genes which may be involved in correct placement of the septum. In *cdc15* mutants, formation of the actin ring is impaired at the restrictive temperature. Thus, septum formation and cytokinesis do not occur and cells become multinucleate. The *cdc15* gene is essential. It encodes a 94 kDa protein whose phosphorylation state and expression change through the cell cycle. In contrast to a *cdc15* mutant, cells lacking *dmf1* (defect in medial fission) are viable and can form a division septum. However, the septum is frequently not placed at the center of the cell. Characterisation of these genes and their products will be presented.

S20-11

TRANSCRIPTION FACTOR AP-1 AND ITS INVOLVEMENT IN APOPTOSIS OF THE MOUSE MAMMARY GLAND

Z. Feng, A. Marti, G. Chicaiza and R. Jaggi. Universität Bern, AKEF, Tiefenaustr. 120, 3004 Bern

Involution of the mouse mammary epithelium is characterized by a restructuring process of the gland and a massive death of epithelial cells by apoptosis. Programmed cell death is associated with a rapid and transient accumulation of a protein kinase A (PKA) activity and of transcription factor AP-1 which is a target of PKA. Upon forced weaning the mammary gland becomes transiently engorged with milk before lobulo-alveolar structures of the gland collapse and cell death occurs. We studied the role of the engorgement and the contribution of lactogenic hormones during lactation and involution. We found that an engorgement in a single gland (by sealing of the gland) is sufficient to induce PKA, AP-1 and several additional markers of programmed cell death including AP-1 dependent matrix-degrading proteases and oligonucleosomal fragmentation of DNA while the other glands of the same animal remain in a lactating state with respect to these parameters. Elevated concentrations of the glucocorticoid hormone dexamethasone (but not prolactin) was sufficient to prevent programmed cell death even in engorged glands and the expression of AP-1 dependent genes such as matrix degrading proteases was strongly inhibited. It is known that hormone-activated glucocorticoid receptor inhibits the activity of AP-1. We postulate that AP-1 is involved in programmed cell death of the involuting mammary gland and that the glucocorticoid receptor functionally interferes with AP-1 function.

S20-12

Nitric oxide induces DNA-fragmentation and apoptotic cell death in glomerular cells

Heiko Mühl, Bernhard Brüne* and Josef Pfeilschifter, Dept. Pharmacology, Biozentrum, Univ. of Basel, Klingelbergstrasse 70, CH-4056 Basel, Switzerland
*Faculty of Biology, University of Konstanz, Germany

We report here that in bovine glomerular endothelial cells and rat glomerular mesangial cells NO donors such as S-nitroso-L-gluthatione, sodium nitroprusside, S-nitroso-N-acetylpenicillamine and spermine NO are able to induce DNA-fragmentation as well as cell death. To detect apoptosis we performed gelelectrophoresis of cytosolic DNA which gave rise to the typical DNA-ladder when NO donors were applied. The results were confirmed and quantitated by the use of an ELISA-based on the detection of cytosolic histone/DNA complexes.

Moreover incubation of mesangial cells with the proinflammatory cytokine IL-1ß which is a potent activator of the inducible NO synthase in these cells also leads to nitric oxide-dependent activation of programmed cell death in mesangial cells.

Taken together these results suggest that nitric oxide released from activated macrophages and mesangial cells may contribute to the tissue damage seen in inflammatory glomerular diseases. They also point to a function of NO in the process of clearence of surplus mesangial cells which is a prerequisite for the recovery of structure and function of the renal glomerulus during the course of disease.

S20-13

IDENTIFICATION OF A SET OF GENES EXPRESSED ONLY IN NORMAL BUT NOT IN TUMORIGENIC CELLS.

Michele Genini, Petra Schwalbe, Beat W. Schäfer, Dept. of Pediatrics, University of Zürich, Steinwiesstr. 75, 8032 Zürich.

Tumorigenesis is a multistep process that has been characterized both by the somatic activation of cellular oncogenes and the somatic or germline inactivation of tumor suppressor genes. Since it was shown by transfer of normal human chromosome that the correction of any single altered suppressor gene is sufficient to achieve full tumorsuppression, the identification of molecules down regulated in tumor cells is of great interest. Such proteins could constitute novel candidate tumor suppressor genes or possible targets of already known suppressor genes, like p53.
We applied a subtractive hybridization procedure between rhabdomyosarcoma cells and human primary myoblasts to isolate genes expressed only in the normal but not in the tumorigenic cells. We identified a wide catalogue of molecules which are down regulated in tumor cells, some of which show no homology with any known proteins. Within this catalogue we chose three known and two unknown molecules for further characterization. The unknown sequences were first used to screen a lambda human myoblast library to isolate the complete cDNAs. The cDNAs are transfected into rhabdomyosarcoma cells to study their effects on tumorigenicity and differentiation.

S20-14

A ROUTINE CLINICAL ASSAY FOR LYMPHOCYTE APOPTOSIS USING FLOW CYTOMETRY

M. Ozsahin, L. Melicharova, N.E.A. Crompton
From the Institute for Medical Radiobiology of the University of Zurich and the Paul Scherrer Institute, CH-5032, Villigen-PSI

We use a flow cytometric evaluation to discriminate different human lymphocytes, and to quantify chemo- or radiotherapeutically induced apoptosis. Following ionizing radiation or incubation with different chemotherapeutical agents like adriamycin, 5-fluorouracil, or etoposide, a part of lymphocytes undergoes apoptosis, which is flow cytometrically demonstrable as a DNA degradation. Lymphocyte subsets, i.e. B-, CT-, Th-lymphocytes or natural killer cells, are first sorted using monoclonal antibodies stained with fluorescent dyes, and the apoptotic cell population is then quantified using a fluorescent DNA stain. The procedure can be conducted within 24 h. There are many situations where a rapid pre-treatment assay of chemo- and radiosensitivity would be welcome. Avoidance of extreme acute or late responses and individualization of patient therapy are included amongst these. This is a rapid assay to determine expected treatment-induced toxicity of normal tissues based on different subsets of lymphocytes, and compares favorably with current techniques requiring weeks.

S20-15

CYTOTOXIC EFFECTS OF BENZIMIDAZOL COMPOUNDS AND X-RAYS IN MOUSE EMBRYOS

M.C. NIEVERGELT-EGIDO*, B. LARSSON* C. MICHEL*
*Institute for Medical Radiobiology (IMR) of the University of Zurich August Forelstr.7, CH-8029

The model blastocyst was choose with respect to the each three dimensional growth.To reach the inner cell mass of blastocyst,drugs must penetrate through the trophectoderm without the aid of capillaries.This may mimic the situation of solid tumours in the clinic. NMRI mouse embryos 72 h(pc)old were treated with X-rays and/or Hoechst 33258 and-33342 compounds in doses(0-3Gy) or concentrations (0.9-9µM). Additional experiments using combinations of Hoechst and irradiation in the dose range were performed.The embryos were cultivated for a total of 5 days.Cytotoxicity of Ho-58 and Ho-42 was studied by a 3h exposure to blastocysts using as morphological endpoints the percentage of embryos which reached eggcylinder stage.For H-58 the growth-inhibition was less than 10% at concentrations of 0.9-2.5µM and the ED50 was obtained with 9.5µM.In conbined experiments (Ho-58+X-rays) the benzimidazol compounds turned out to be protective.After treatment of the blastocysts with Ho-42(0.9-9 µM).ED50 was found with 3µM. The combined treatment exhibited a sensitizing effect.

S20-16

Analysis of programmed cell death during metamorphosis and isolation of the bcl-2 homologue from *Xenopus laevis*

Franziska Kuhn, Bettina Tilton and Daniel Schümperli
Zoologisches Intstitut, Abteilung für Entwicklungsbiologie
Baltzerstr. 4, 3012 Bern

During amphibian metamorphosis, many larval tissues are replaced by adult structures. In particular, tail regression in frogs is a well established example of programmed cell death (PCD). We have investigated PCD during metamorphosis in *Xenopus laevis*. In a first approach, we have analysed if tail regression involves the well-defined apoptotic type of cell death. We used electron- and light-microscopy to define morphological characteristics and DNA laddering experiments to characterize a biochemical marker of apoptosis. A second approach involved cloning and sequencing of a 160 bp fragment of the bcl-2 gene from *Xenopus laevis*. This 24 kD integral membrane protein was first characterized in human B cell lymphomas and has been shown to prevent apoptosis in many cell types. We plan to further investigate the expression of the *Xenopus* bcl-2 gene during metamorphosis and to characterize its genomic organisation.

S20-17

A TRANSPLASMA MEMBRANE-OXYDOREDUCTASE IS INDUCED DURING THE G1 AND G2/M-PHASES OF THE CELL CYCLE

R. Zurbriggen and J.-L. Dreyer, Institut de Biochimie, Université de Fribourg, CH-1700 Fribourg

The presence of a transplasma membrane redox system is well established but its function is not well defined so far. Transplasma membrane Oxydoreductases (PMO's) probably act as redox sensors at the plasma membrane upon oxydative stress and play a significant function in various cell activation processes. Our studies on PMO's in a NB41A3, neuroblastoma cell line, shows that PMO's activities changes upon cell development and growth. Elutriation studies show that the enzyme is mainly active during the G1 and during the G2/M-phases of the cell cylce. a-Amanitin, a specific inhibitor for RNA polymerase-II, which blocks growth in the G1-phase, induces a 3-fold increase of PMO's activity. Taxol, an specific inhibitor for both the anaphase and the metaphase, induces an 43% activity increase. These results were further confirmed by means of FACS analysis. In addition similar studies were undertaken with N-Tera cells, a neuroblastoma cell line which can be differentiated to mature neurons in the presence of 10mM retinoic acid. N-tera cells whose differentiation has been induced by retinoic acid but blocked in the G0-phase display an activity drop of >50%. These data corroborate that PMO's play a significant role in cell differentiation and growth, mainly in the G1 and G2/M phases.

S20-18

Cell cycle arrest by tyrosine kinase Abl involves altered early mitogenic response

T.Mattioni & .O.Bchini Hooft van Huijsduijnen, and D.Picard Département de Biologie Cellulaire, Université de Genève, CH-1211 Genève 4

Activated forms of the nuclear and cytoplasmic tyrosine kinase c-Abl are completely cytoplasmic and oncogenic. The overexpression of c-Abl, and in certain fibroblast cell lines even of v-Abl, leads to a cell cycle arrest revealing an alternative function of Abl. To facilitate the analysis of this growth inhibitory function we have taken advantage of regulable Abl-estrogen receptor (ABL:ER) fusion proteins. Oncogenic in the presence of estrogen, they are reversibly switched to inhibit cell proliferation upon removal of hormone. Using this system, we demonstrate that hypophosphorylated Abl derivatives with low kinase activity inhibit growth. Since an almost exclusively cytoplasmic ABL:ER protein is fully growth inhibitory, growth inhibitory interactions may occur in the cytoplasm. We identify the cell cycle arrest as an early G1 or G0-like block. Interestingly, growth inhibition correlates with an altered expression pattern of early serum response genes; c-Jun mRNA and c-Fos protein levels are elevated in Abl-blocked cells. In view of the two functional modes of overexpressed Abl proteins, one can speculate that normal c-Abl may be involved in relaying growth regulatory signals from the membrane to the nucleus, implying the involvement of other protein-partners. For this reason, we decided to screen a c-DNA library using a Two-Hybrid-System. One hybrid is a fusion between the LexA DNA-binding domain (aa1 to 211) and CIV-Abl (aa 48 to 630), with an inactive tyrosine kinase (mutation K290M). The second hybrid is a fusion between VP16 acidic activation domain and a protein from the library.

S20-19

ECTO-γ- GLUTAMYLTRANSFERASE ACTIVITY AND SUSCEPTIBILITY TO APOPTOTIC CELL DEATH IN A MDR$^+$ T CELL LINE

Riccardo Graber*, Vladimir von Fliedner‡, Gabriele A. Losa*
*Laboratorio di Patologia Cellulare, Istituto Cantonale di Patologia, 6600 Locarno,
‡Centre Pluridisciplinaire, 1000 Lausanne, Switzerland.

The enzyme γ-glutamyltransferase (γ-GT) catalyzes the first step in the extracellular transpeptidation of the cellular glutathione into amino-acid intermediates which are subsequently transported across the cell membrane. Many studies have shown that γ-GT was elevated in various tumors, enhanced in dexamethasone-induced apoptotic CEM cells and, found modulated in drug-resistance. The γ-GT activity and their kinetic properties were analyzed in two human T-lymphoblastoid cell lines, one sensitive (CCRF-CEM cells) and the other resistant (VBL-100 CEM cells) to vinblastin. Resistant VBL-100 CEM cells, unlike CCRF-CEM subclone, reacted with monoclonal antibodies directed against external epitopes of the MDR1 gene product, P-gp and exhibited a γ-GT activity higher (3.6 nmol/min) than the sensitive subclone (2.4 nmole/min) devoid of P-gp. In contrast, substrate affinity (Michaelis-Menten analysis) was found similar (Km=1.0 mM). Acivicin, a specific inhibitor of γ-GT activity, induced a dose-dependent growth inhibition of both cell lines, but its apoptotic effect at 10^{-6} M was more marked than that of dexamethasone, a known inducer of apoptosis in CEM cells. When compared to CCRF cells, the VBL-100 resistant subclone showed a higher degree of γ-GT inhibition by acivicin and also an increased level of apoptosis. Our results suggest that drug-resistant VBL-100 CEM cells, bearing the MDR1-encoded efflux system P-gp, also exhibit an increased membrane γ-GT complex which might be related to an active influx system and to an increased susceptibility to apoptotic cell death.

Cellular and molecular biology of epithelial transport

S21-01

The modulation of the epithelial phenotype correlates with changes in proteinase expression

Janos Peli, Martin Oft and Ernst Reichmann
Swiss Institute For Experimental Cancer Research (ISREC)

We are studying the effects of certain tumor-relevant oncoproteins on the development and differentiation of mammary epithelium in vivo. As one model-oncogene the v-Ha-*ras* gene was introduced into non-tumorigenic mouse mammary epithelial cells. When injected into recipient mice these Ras-transformed cells caused highly vascularized, rapidly growing tumors in which the injected epithelial cells underwent dramatic changes in phenotype. Three days after injection the Ras-expressing cells had developed into differentiated, lumen-forming structures. When re-isolated and analyzed, these cells still exhibited epithelial properties. In contrast, cells recovered from full-blown tumors (20-30 days after injection) were fibroblastiod in shape, had lost many of their epithelial characteristics, expressed certain mesenchymal proteins and readily invaded adjacent muscle and mesenchymal tissues. In addition these cells expressed distinct proteinases such as urokinase (uPA), tissue plasminogen activator (tPA), stromelysin, and interstitial collagenase at distinct locations within the tumors. Preliminary data suggest that the pattern of proteinase expression correlates with the distribution of distinct cell phenotypes. These findings indicate that a malignant cancer was generated. Its development was accompanied by clear changes in tumor cell phenotype. These changes in phenotype seem to correlate with changes in proteinase (and proteinase-inhibitor) expression and most likely with tumor cell invasiveness.

S21-02

FLUORESCENCE MICROSCOPIC STUDY OF THE Na^+ CONCENTRATION IN THE LATERAL INTERCELLULAR SPACES (LIS) OF EPITHELIAL CELLS

Chatton, J.-Y., Spring, K.R. National Institutes of Health, Bethesda, MD 20892 - USA

MDCK cell monolayers grown on glass coverslips were used to examined the Na^+ concentration in individual LIS by video fluorescence microscopy. The LIS was loaded with the Na^+-sensitive fluorescent dye SBFO by incubation of the monolayers for 75-90 min with 250 µM of the membrane impermeant form of the dye. After dye loading, the monolayers were perfused at 37°C with solutions buffered with HEPES or bicarbonate/CO_2 containing 142 mM Na^+. Ratios of the fluorescence images after sequential excitation with 340 and 380 nm light were performed; ***in situ*** calibration of the dye was accomplished by equilibrating LIS Na^+ with perfusate Na^+ after blocking the Na^+ pump with $5 \cdot 10^{-4}$M ouabain. Measurements of Na^+ along the basolateral-to-apical axis of the LIS at 1.0 or 1.5 µm intervals did not reveal a Na^+ gradient when the perfusate was either HEPES or bicarbonate/CO_2 solutions. In bicarbonate solutions, the mean Na^+ concentration (mM) was 157.2±2.3 (27 LIS, 10 expts), ~15 mM higher than the bath Na^+ concentration. In HEPES solutions, however, the Na^+ concentration was not different from the bath concentration (142.7±3.1). In conclusion, Na^+ was at higher concentrations in the LIS than in the bathing media when bicarbonate was present and was not in the absence of bicarbonate. In either situation, Na^+ concentration gradients were not found.

S21-03

EFFECTS OF TGFβ ON HUMAN BRONCHIAL EPITHELIAL CELLS (BEAS-2B) TRANSFECTED WITH THE INDUCIBLE NITRIC OXIDE SYNTHASE GENE

E. Felley-Bosco, Institut de Pharmacologie et Toxicologie, UNIL, Bugnon 27, Lausanne

Increased expression of the inducible nitric oxide synthase (iNOS) has been observed in bronchial epithelial cells of asthmatic patients. To assess the effect of nitric oxide (NO) in bronchial epithelial cells we developed an in vitro system by transfection of *iNOS* gene into BEAS-2B cells (Am.J. Respir. Cell. Mol. Biol.11: 159-164, 1994). BEAS-2B cells, like normal human bronchial epithelial cells, are sensitive to TGFβ, which induces irreversible growth arrest when cells are grown at low density. However, in *iNOS* transfected BEAS-2B (2B-nos3) exposed to 4 pM TGFβ we observed an increased colony formation efficiency (115% of control) while in the control vector transfected cell line (2B-cmv2-1) the colony formation efficiency was decreased by TGFβ (33% of control) as expected. The different response to TGFβ was not due to a different binding capacity between 2b-cmv2-1 and 2b-nos3 since in both lines the number of receptors/cell was the same (1855±412 vs 1794±740 in 2bcmv2-1 and 2b-nos3, respectively). Furthermore, cross-linking experiment using radiolabelled TGFβ showed that both cell lines express similar amount of TGFβ receptors I, II and III. In addition TGFβ induced plasminogen activator inhibitor (PAI-1) expression was higher in 2B-nos3 than in 2B-cmv2-1 (iNOS/GAPDH = 3.04 vs 1.96, respectively). In conclusion, some of the effects of TGFβ on cultured human bronchial epithelial cells are altered in presence of continuous iNOS expression.

S21-04

MURINE AUTOIMMUNE GASTRITIS AND H,K-ATPASE

Claeys, D.[1], Saraga, E.[2], Fischer, G.[1], Rossier, B.C.[3] and Kraehenbuhl, J.-P.[1]
Inst. of Biochemistry[1], Inst. of Pathology[2] and Inst. of Pharmacology[3], University of Lausanne .

Autoimmune gastritis (AIG) is accompanied by autoantibodies to the gastric H,K-ATPase. Neonatal thymectomy of BALB.D2 mice induces AIG. Only the autoimmune sera immunoprecipitate the α and β subunits of the H,K-ATPase expressed in *Xenopus* oocytes. The role of the H,K-ATPase and auto-antibodies in AIG is unknown. Immunisation of BALB.D2 mice with gastric membranes enriched in H,K-ATPase triggers AIG. The role of each of the proton-pump subunits in the induction of AIG was studied using this model. Murine AIG appears to be $CD4^+$ T cell mediated. The inflammatory lesions consist mainly of $CD4^+$ T cells. CD8αα T cells are also abundant. We analysed the presence of potential autoreactive $V\beta6^+$, $V\beta11^+$ T cells in the gastric mucosa by immunocytochemistry.

S21-05

LOCALIZATION OF Na CHANNELS IN RENAL COLLECTING DUCTS: EFFECT OF ELEVATED SERUM ALDOSTERONE

Ernst, S.A., Duc, C., Canessa, C.M., and Rossier, B.C. Institut de Pharmacologie, Université de Lausanne, Lausanne, Switzerland

The highly selective, amiloride-sensitive epithelial Na channel (ENaC) forms the rate limiting step for Na reabsorption in the distal nephron and is regulated by aldosterone. The rat channel (rENaC) was cloned recently and shown to consist of 3 homologous subunits. Specific antibodies were used to determine the distribution of α and β subunits in cortical, outer and inner medullary collecting ducts (CCD, OMCD, IMCD) from normal rats, and from rats maintained on a low salt (LS) diet for 7 days to maximally elevate serum aldosterone levels. In CCD and OMCD from normal rats, immunoperoxidase staining for the β subunit at the light and EM levels was distributed in the cytosol of principal cells in an apparently non-polarized manner. In striking contrast, β subunit staining in CCD and OMCD from LS animals was restricted to apical surfaces with little cytoplasmic reactivity. The localization of α-subunit in these segments was similarly restricted to apical membranes in the LS condition, whereas staining was absent in controls. With both subunit antibodies, intercalated cells in CCD and OMCD were unreactive, and IMCD were unstained. The results suggest that aldosterone promotes accumulation of ENaC at the apical membrane and are entirely consistent with patch clamp studies (Pacha, et al., 1993) which showed that ENaC activity was absent in apical CCD membranes of normal rats, but was induced in LS animals primarily by an increase in channel number.

S21-06

CONTROL OF MINERALOCORTICOID-DEPENDENT SODIUM TRANSPORT IN THE EPITHELIAL A6 KIDNEY CELL LINE.

Anne May, Alessandro Puoti and Bernard C. Rossier
Institut de Pharmacologie et de Toxicologie, Université de Lausanne, 1005 Lausanne, Switzerland

The highly selective, amiloride sensitive sodium channel found at the apical membrane of epithelial cells constitutes the rate-limiting step of sodium reabsorption. This reabsorption process is tightly regulated by mineralocorticoids such as aldosterone and vasopressin, which are known to increase the transepithelial transport of sodium. However, the molecular mechanisms responsible for this upregulation of sodium transport are still largely unknown.

The three subunits of the epithelial sodium channel have recently been cloned in the epithelial A6 cell line, derived from *Xenopus laevis* kidney. Since this cell line responds to mineralocorticoids, it represents a very fruitful model for the investigation *in vitro* of the mechanisms of aldosterone-dependent regulation of sodium reabsorption.

We are investigating the level of transcription (by Northern analysis) and of translation (by immuno-precipitation and immunocytochemistry) of the three channel subunits in response to aldosterone. Time-course experiments showed that in A6 cells cultured on petri dishes, the aldosterone-dependent upregulation of mRNA abundance is different for α, β and γxENaC. Polyclonal antibodies are now being raised against each of the three subunits.

S21-07

CLONING AND FUNCTIONAL ANALYSIS OF THE AMPHIBIAN RENAL EPITHELIAL SODIUM CHANNEL

Alessandro Puoti, Anne May, Cecilia M. Canessa, Jean-Daniel Horisberger, Laurent Schild and Bernard C. Rossier. Institut de Pharmacologie et de Toxicologie, Université de Lausanne, Switzerland

In epithelia, the rate-limiting step for sodium reabsorption occurs via an amiloride-sensitive sodium channel. This channel is also expressed in the A6 epithelial cell line derived from *Xenopus* kidney. We cloned three full-length cDNAs coding for three homologous proteins termed α, β and γxENaC (standing for *Xenopus* Epithelial sodium Channel). Transcripts of xENaC were detected in aldosterone-stimulated A6 cells grown on Petri dishes, in *Xenopus* kidney, lung and in less amounts in stomach and skin.

Coexpression of α, β and γxENaC in *X. laevis* oocytes led to functional sodium channels with biophysical properties indistinguishable from the native sodium channel in A6 cells.

We also cloned two additional subunits, termed β_2xENaC and γ_2xENaC, in view of their high degree of similarity to β and γxENaC. In some tissues, the distribution of β_2xENaC and γ_2xENaC transcripts is different from that of β and γxENaC. Functional expression of α, β and γ_2xENaC indicates that the γ_2xENaC subunit is responsible for a sodium channel with different electrophysiological properties than those formed by α, β and γxENaC. γ/γ_2xENaC chimaeras should allow to identify domains of functional importance.

S21-08

A CHANNEL-LIKE CONFIGURATION OF THE NA,K-PUMP IS PERMEABLE TO PROTON.

Wang X. and Horisberger J.-D., Institut de Pharmacologie et de Toxicologie, Université de Lausanne.

The Na,K-pump physiologically performs ATP-driven Na for K exchange. In the absence of external cations, it has been shown to work in the «uncoupled Na efflux mode» which is an electroneutral Na/anion cotransport or possibly a Na/proton exchange. Using sodium-loaded *Xenopus* oocytes in which Na,K-pumps were overexpressed by injection of cRNA of the *Xenopus* Na,K-pump α and β subunits, we observed a strongly inward rectifying and pH dependent ouabain-sensitive current. We demonstrated a ouabain-sensitive intracellular acidification related to the ouabain-sensitive current indicating that this current was carried by protons. The reversal potential of the ouabain-sensitive current was dependent on external pH as expected for a proton conductive pathway. We conclude that in the absence of external K the Na,K-pump can mediate a large inward electrogenic transport of proton, as if its E2 conformation was permeable to proton.

S21-09

ALDOSTERONE INCREASES SODIUM SENSITIVITY OF Na,K-ATPase CONTAINING AN α1 SUBUNIT IN A6 KIDNEY CELL EPITHELIA.

Beron, J. and Verrey, F., Institute of Physiology, University of Zurich.

Aldosterone (10^{-6} M) leads to an early *in situ* activation of Na,K-ATPase function at the surface of A6 cells, as previously shown by measurements of ouabain binding rates and cell surface-labeled enzyme. We now measured the effect of aldosterone on the cardiotonic steroid-sensitive pump current (Ip) in A6 monolayers, which had been apically permeabilized with amphotericin B. Short-term aldosterone treatments (2.5 hours) did not affect the maximal Ip at 90 mM Na, nor the Na concentration required for half maximal activation. In contrast, significant differences in Ip were observed at low intracellular Na concentrations between control and aldosterone treated cells (% increase: 70±12 at 2.5 mM; 66±9 at 5 mM; 29±4 at 10 mM). Whether this increase in Ip was due to the activation of Na pumps containing the α1 subunit was tested with transfected A6 cell lines stably expressing the α1 subunit of *B. marinus* (α1 TBM). This subunit conferred a higher level of cardiotonic steroid resistance to hybrid pumps compared to the endogenous ones. In some of the cell lines the expression level of α1 TBM was enhanced by a pretreatment with Na butyrate as determined by Western blotting and measurement of the cardiotonic steroid-resistant Ip. Aldosterone increased the Ip (at 5 mM Na) carried by the hybrid pumps to a similar extent as that of endogenous pumps. These results show that aldosterone (10^{-6} M) increases, at physiological intracellular Na concentrations, the turnover rate of pumps containing an α1 subunit.

S21-10

IDENTIFICATION OF EARLY ALDOSTERONE REGULATED mRNAs IN A6 KIDNEY CELLS BY DIFFERENTIAL DISPLAY

Spindler, B. and Verrey, F., Physiologisches Institut der Universität Zürich, CH-8057 Zürich

Transcellular sodium reabsorption across epithelia of A6 distal nephron cells is stimulated by aldosterone (10^{-6} M). This increase in transcellular transport starts after a lag period of approx. 45 min, and results from the activation of the apical epithelial Na channel and the basolateral Na,K-ATPase. This activation depends on ongoing transcription and translation and is thought to be mediated by the action of transcriptionally regulated mediator(s). The aim of this study is to identify cDNAs corresponding to acutely regulated genes which will later be tested for their potential role in transport regulation. To visualize the hormone action on a large number of mRNAs we used a modification of the differential display reverse transcription PCR (DD-PCR) method. We visualized approximately 6000 bands corresponding to mRNAs from two sets of control and aldosterone (40 min) treated epithelia. Those bands which showed a roughly 2-fold or larger change in intensity for both sets of RNAs were considered as reproducibly regulated. Approximately 40 bands corresponding to 0.7% of the total number of bands fulfilled these arbitrary criteria. Slightly more than half of these bands were up- and the others down-regulated. Selected cDNA bands were reamplified and cloned. To identify the "regulated" cDNA among the cloned fragments, pairs of Southern blots were performed using the control and test DD-PCR as probes. The corresponding mRNAs were then visualized and their regulation confirmed by Northern blotting. We conclude that approximately 0.7% of the mRNAs expressed in A6 cells are acutely regulated by aldosterone and represent potential mediators of Na transport activation.

S21-11

CYTOSKELETAL DISRUPTION IN A6 CELLS: INHIBITION OF ADH-INDUCED EXOCYTOSIS AND Na CONDUCTANCE

Verrey, F.*, Groscurth, P.# and Bolliger, U.*, Institutes of *Physiology and #Anatomy, University of Zürich

Antidiuretic hormone (ADH) produces a stimulation of apical fluid phase endocytosis, protein secretion and NaCl reabsorption in *Xenopus laevis* A6 distal nephron cell epithelia pretreated with aldosterone (10^{-6} M). The increase in NaCl transport is mediated by a sequential opening of apical Cl and Na conductances. The aim of this study was to characterize the actin and tubulin cytoskeleton of A6 cells and to assess the impact of its disruption on baseline and ADH-induced apical vesicular membrane movements and ion transport in order to test for possible functional links. The microfilament (MF) and microtubule (MT) networks and their disruption by cytochalasin D and cold plus nocodazole, respectively, were visualized by confocal laser microscopy. MF disruption inhibited the ADH-induced protein secretion (exocytic movements) (minus 35%) without affecting the ADH-induced endocytic movements. In the case of MT disruption, the ADH-induced stimulation of protein secretion and fluid phase endocytosis was decreased by 70 and 44%, respectively. At the ion transport level, MF and MT disruption only unsignificantly affected the ADH-induced Cl conductance, while they decreased the ADH-induced stimulation of Na transport (amiloride-sensitive conductance) by a factor of 2 to 4. Taken together these data support the hypothesis that the modulation of Na channel expression by apical vesicular membrane movements plays a role in Na transport regulation by ADH, in contrast to the regulation of the apical Cl conductance by the same hormone.

S21-12

ROLE OF NITRIC OXIDE IN THE HYPOXEMIA-INDUCED RENAL DYSFUNCTION OF THE NEWBORN RABBIT

L. Ballèvre, M. Thonney, D. Mosig, JP. Guignard
Service de pédiatrie, CHUV, Lausanne, Switzerland.

The present study was performed in anesthetized and mechanically-ventilated newborn rabbits to define whether endogenous NO is involved in the pathogenesis of the hypoxemia-induced renal dysfunction. Arterial blood pressure (MAP) and heart rate were continuously monitored. Each animal acts as is own control. In group 1 (n = 9), acute hypoxemia (PaO_2 = 43 mm Hg) was induced within 45 to 60 min and a 60-min clearance was thereafter performed. Hypoxemia significantly decreased renal blood flow (RBF) by 17 ± 7 %, glomerular filtration rate (GFR) by 11 ± 6 % and MAP by 11 ± 2 %. A second group of 9 animals was administered L-NAME, a NO synthesis inhibitor, to determine the role of NO in regulating the hemodynamics of the immature kidney in physiological conditions. L-NAME significantly increased the renal vascular resistance (RVR) by 31 ± 9 % and decreased RBF and GFR by 20 ± 6 % and 13 ± 5 %, respectively. Acute hypoxemia (PaO_2 = 45 mm Hg) was induced in 12 additional newborn rabbits during L-NAME infusion in order to define the role of NO in the renal hypoxemia-induced dysfunction. The alterations observed in group 3 (L-NAME + Hypoxemia) were greater than in group 1 (Hypoxemia). The present results suggest that (1) endogenous NO plays a significant role in maintaining the basal perfusion of the immature kidney; (2) NO synthesis is not altered by acute hypoxemia and is opposing to the hypoxemia-induced renal dysfunction of the immature newborn kidney.

S21-13

URATE AND P-AMINOHIPPURATE TRANSPORT BY HUMAN BASOLATERAL MEMBRANE VESICLES (BLMV) OF PROXIMAL TUBULES.

Guisan, B. and Roch-Ramel, F. *Institut de Pharmacologie et de Toxicologie de l'Université, CH-1005 Lausanne.*

50µM 14[C]p-aminohippurate (PAH) uptake by human BLMV was measured in the presence of an inward 100 mM NaCl gradient, with or without addition of 10 µM oxoglutarate to the uptake medium. 15 sec PAH uptakes were respectively 81±12 and 23±4 pmol/mg protein (n=3), the mean equilibrium uptake being 52 pmol/mg protein. Thus, concentrative PAH uptake was energized by oxoglutarate sodium cotransport. As in rats and pigs, the NaCl gradient could not be replaced by sodium-gluconate gradient. 50 µM 14[C]-urate uptake was not stimulated by such inward oriented NaCl gradient, with or without oxoglutarate in the uptake medium. It was stimulated by an inside positive potential created by an inward oriented 100 mM K-gluconate gradient plus valinomycin. 15 sec 14[C]-urate uptake was 81±9 compared to 30±5 pmol/mg protein in control (K-gluconate gradient,no valinomycin), the mean uptake at equilibrium being 59 pmol/mg prot. No urate-chloride exchange was observed. In vivo, because of cell negativity, this transport mechanism should drive urate from cell to interstitium, the second step in tubular reabsorption. Basolateral urate tansport in the human is similar to that in rats, which reabsorbes urate, but differs from that in pigs which secretes urate.

S21-14

EFFECT OF URICOSURIC DRUGS AND PYRAZINOATE ON URATE UPTAKE BY HUMAN BRUSH-BORDER MEMBRANE VESICLES (BBMV).

Roch-Ramel, F. and Guisan, B. *Institut de Pharmacologie et de Toxicologie de l'Université, CH-1005 Lausanne.*

Urate renal reabsorption in human is mediated by a urate-anion exchanger localized in brush-border membranes. The cis-inhibition of urate uptake by uricosuric drugs was investigated in human BBMV loaded with 5 mM lactate. In absence of drugs, 50 µM [14-C]urate 15 sec uptakes were 29±3 and 54±9 pmol/mg protein, in unloaded and lactate loaded BBMV, respectively. Thus, [14-C]urate/lactate uptake was 25±4 pmol/mg protein. 50 % inhibition of [14-C]urate/lactate uptake was obtained with 2 µM benzbromarone, 14 µM losartan, and 130 µM probenecid. This demonstrate that uricosuric drugs can decrease urate reabsorption by inhibiting urate transport by the urate-anion exchanger. In contrast,100 µM pyrazinoate (an antiuricosuric metabolite of pyrazinamide) stimulated 15 sec [14-C]urate/lactate uptake, which was 52 pmol/mg protein. At 1 mM, pyrazinoate inhibited [14-C]urate/lactate uptake by 40 %. The stimulatory effect of PZA suggests the existence of two anion-exchangers, one for which lactate and PZA have more affinity than urate, and another one for which urate and PZA have more affinity than lactate. At 100 µM, PZA was transstimulated by lactate, and in turn PZA transstimulated urate uptake. At 1 mM, PZA competed with urate for exchange with lactate.

S21-15

CHOLESTERYL OLEATE ABSORPTION BY SMALL INTESTINAL BRUSH BORDER MEMBRANE

S. Compassi, H. Hauser and G. Schulthess

Laboratorium für Biochemie IV, Eidgenössische Technische Hochschule Zürich, ETH-Zentrum, Switzerland

The kinetics of cholesteryl oleate absorption by the brush border membrane were investigated using both bile salt micelles and phospholipid unilamellar vesicles as the donor. Cholesteryl oleyl ether was used as a nonhydrolyzable model for cholesteryl oleate. As in-vitro models rabbit jejunal brush border membrane vesicles and intact enterocytes isolated from pig jejunum were employed. We are able to show that intact cholesteryl esters are efficiently absorbed by the brush border membrane and that this process is protein-mediated. Supernate proteins released from brush border membrane vesicles are shown to catalyze the exchange of both free and esterified cholesterol between two populations of phospholipid unilamellar vesicles. Our results challenge the generally accepted view that cholesteryl esters have to be hydrolyzed prior to absorption by the brush border membrane.

S21-16

CHOLESTEROL UPTAKE IN THE SMALL INTESTINE

Franz E. Weber, Dario Boffelli, Moritz Werder and Helmut Hauser, Laboratorium für Biochemie IV, Eidgenössische Technische Hochschule Zürich, ETH-Zentrum, Switzerland.

Contrary to textbook opinion Thurnhofer and Hauser (1990) have shown that uptake of cholesterol by small intestinal brush border membrane vesicles is protein-mediated. Due to the lack of any sequence information for proteins which are involved in lipid uptake only functional reconstitution or expression cloning can lead to the isolation of the protein or its cDNA. Expression cloning in oocytes from Xenopus laevis was our first choice. Oocytes injected with mRNA from the duodenum exhibited an increase in cholesterol uptake by 25-40%. This result proves the existence of a protein which mediates cholesterol uptake. Unfortunately an 40% increase is too small for a successful screening of a cDNA library. In an attempt to purify the protein, brush border membrane vesicles were solubilized with a short chain lecithin and cholesterol exchange activity was successfully reconstituted in artificial liposomes. Different parameters affecting this process were investigated, namely the effect of ionic strength, pH and the presence of different lipids in the reconstituted proteoliposomes. This system seems suitable to partially purify the above-mentioned activity.

S21-17

TARGETING OF T-CADHERIN AND N-CADHERIN IN POLARIZED EPITHELIAL CELLS

Koller E. and Ranscht B.,La Jolla Cancer Res.Fdn., 10901 N. Torrey Pines Rd., La Jolla, CA 92037

In polarized epithelial cells, classical cadherins containing the conserved cytoplasmic region are segregated to the basolateral membrane domain, while GPI-linked membrane molecules are targeted apically. MDCK cells were stably transfected with chick T-cadherin cDNA. T-cadherin, a GPI-linked molecule, is targeted to the apical side in MDCK cells. In contrast, transfected chick N-cadherin is segregated basolaterally. T/N-cadherin chimeras were constructed and examined for their specific targeting. A N-cadherin mutant (N/T_{GPI}) containing the extracellular portion of N-cadherin, part of the T-cadherin EC5 domain and the GPI-anchor signal sequence, was targeted apically. In contrast, NΔcyt, a deletion mutant of N-cadherin without the cytoplasmic domain, was localized basolaterally. Thus, the cadherin cytoplasmic region which associates with the cytoskeleton is not sufficient for basolateral targeting. Our results suggest that an amino acid stretch located within the carboxyterminal portion of EC5 and the hydrophobic domain is crucial in targeting T- and N-cadherin to distinct membrane domains.

S21-18

EFFECTS OF HYPER AND HYPOTHERMIA ON RENAL FUNCTION IN NEWBORN RABBITS

P. Gilliéron, M. Thonney, D. Mosig, J.-P. Guignard
Service de pédiatrie, CHUV, Lausanne, Switzerland.

The effect of mild hyper-and hypothermia (± 2°C) was investigated in three groups of 9 anesthetized and mechanically-ventilated newborn rabbits. Glomerular filtration rate (GFR) and renal blood flow (RBF) were assessed by the clearance of inulin and para-aminohippuric acid. **Hyperthermia** induced a significant increase in diuresis (+45%), sodium (+171%), potassium (+118%), and chloride (+109%), respectively. There changes were associated with an increase in GFR (+16 ± 5%; $p<0.01$) in spite of an increase in the renal vascular resistance (+15 ± 4%; $p<0.01$) and a decrease in RBF (-10 ± 3%; $p<0.01$). This finding suggests that hyperthermia induces predominant efferent arteriolar vasoconstriction. Pretreatment with perindoprilate, an angiotensin converting enzyme inhibitor, completely blunted the phenomenon, indicating that angiotensin II plays a crucial role in the response to hyperthermia. **Hypothermia** induced a fall in diuresis (-18%), potassium (-61%) and chloride (-19%) excretion, and a rise in sodium excretion (+40%). GFR and RBF both decreased significantly by 20 ± 5% ($p<0.05$) and 31 ± 4% ($p<0.05$), respectively. Intense vasoconstriction and decreased active soluble transport explain the changes induced by mild hypothermia.

Authors Index

Abramowski, D. S06-12
Acha-Orbea, H. S16-30, S16-31
Ackermann, M. S03-22, S05-33, S05-61
Ackermann, R. S12-17
Adlkofer, K. S03-10
Aebi, M. S10-05, S16-07
Aebischer, P. S03-01, S17-19, S17-20
Affolter, M. S05-55, S06-04, S12-22
Agostoni, E. S05-53
Aguet, M. S03-20
Aguzzi, A. S03-10, S03-16, S03-18, S03-34
Alberati-Giani, D. S17-25
Alberi, S. S07-31
Albrecht, D. S06-31
Albrecht, U. S07-10
Alcedo, J. S12-19
Alevizopoulos, A. S06-43
Alliod, C. S07-32
Almaraz, L. S01-08
Altermatt, H.J. S13-18
Althaus, F.R. S05-40, S05-41
Altmann, M. S19-04
Amacker, M. S05-14, S05-15
Aminian, K. S16-38
Amrhein, N. S04-07
Amstutz, H. S16-11
Anagli, J. S11-04, S11-18, S11-28
Andres, A.-C. S06-31, S06-32, S06-34, S06-64, S13-18
Andressen, C. S11-15
Anliker, M. S05-23
Appel, F. S14-29
Arcaro, A. S06-35
Arigoni, M. S03-34
Arni, S. S14-15
Arnold, A. S13-18
Arrighi, J.-F. S08-09
Arsenijevic, D. S16-18
Arthur, B.I. S12-14
Ashburner, M. S05-31
Avison, M. S06-03

Bachmann, E. S03-03
Backes, S. S19-08
Bade, E.G. S13-09
Bader, C.R. S03-31, S03-32, S03-33
Baetge, E. S17-19, S17-20
Bähler, J. S05-07
Baier-Kusterman, W. S01-06
Bajo, V. S17-22
Balazs, R. S17-26
Balerna, M. S16-41
Ballèvre, L. S21-12
Ballmer-Hofer, K. S06-29, S06-30, S06-33, S20-06
Banach, K. S14-19
Bandtlow, C.E. S07-30
Bankoul, S. S17-15
Bantle, St. S16-20
Barakat Walter, I. S07-19
Barbey, C. S03-05
Barbier-Brygoo, H. S04-05
Baroffio, A. S03-31, S03-32, S03-33
Baron, A. S06-58
Bartholdi, D. S07-28
Bashkirov, V. S05-11
Basler, K. S06-04
Batra, S. S01-09
Bauer, C. S01-01, S01-05, S01-06, S01-07, S01-12
Baumann, M. S16-43
Baumgartner, S. S12-03
Baur, H. S04-21
Baur, M. S05-05
Bchini-Hooft van Huijsduijnen, O. S20-18
Beard, P. S05-37, S05-38
Bednar, J. S05-28
Beermann, F. S03-15, S07-17
Beffa, R.S. S04-24
Beggah, A.T. S06-23, S10-08, S10-09
Beguin, P. S06-23, S10-08
Belet, M. S11-21
Belli, S. S05-44
Bello, B. S12-21
Benghezal, M. S10-01
Benke, D. S03-34, S17-10, S17-26
Bennet, R.J. S05-30
Bennoun, P. S04-29
Benson, J. S03-34
Bény, J.-L. S06-58
Benz, B. S17-11
Benzi, R. S16-18
Berchtold, M.W. S05-19, S11-03, S11-12, S11-13, S11-15, S11-22
Berdoz, J. S16-28
Berg, P. S03-13
Berger, E.G. S10-11, S10-12
Bernard, V. S04-36
Bernasconi, K. S03-11
Bernheim, L. S03-32
Beron, J. S21-09
Bertin, S. S06-57
Beug, H. S13-22
Beyeler, M. S04-21
Bi, X.-N. S17-04
Bichet, S. S01-06
Bichsel, V. S06-40
Bickel, M. S06-05, S06-09, S06-40
Bider, M.D. S14-26
Bieber, A. S12-08
Billeter, R. S05-25, S06-45
Binz, T. S04-16
Binzoni, T. S01-04
Bittar, P.G. S17-13
Blake, D.J. S14-25
Bluethmann, H. S03-20, S06-13, S17-21
Blum, S. S16-08
Blum-Tirouvanziam, U. S18-08
Boelsterli, U. S16-21
Boffelli, D. S21-16
Bogumil, R. S01-02, S03-07
Bollag, O. S11-19
Boller, T. S04-07, S04-32
Bolliger, U. S21-11
Bonny, C. S08-10
Boothroyd, J.C. S18-01
Bopp, D. S12-13, S12-14
Bormann, P. S07-11
Borner, C. S20-02, S20-03, S20-04, S20-05
Bornmann, C. S05-32
Borroni, E. S17-21
Borsig, L. S10-11
Bös, M. S02-03
Boschetti, A. S04-22, S04-23
Boscoboinik, D. S06-36
Bosshard, G. S16-35
Botreau, M. S09-03
Botteri, F. S07-09
Bouras, C. S17-13
Boutellier, U. S16-37
Boutin, J.A. S06-57
Bovet, L. S04-09
Bovia, F. S03-28
Bowen, B. S10-11
Brack, C. S12-17
Braconi Quintaje, S. S06-24, S06-27
Braguglia, D. S05-42
Brandenberger, M. S16-37
Brändle, U. S07-15
Brandner, S. S03-16, S03-18
Breiling, A. S05-01
Brendel, K. S14-35
Brenner, H.R. S07-23
Brentrup, D. S12-08
Brenz Verca, S. S03-09
Briggs, L.J. S06-28
Britt, J.C. S07-23
Brodbeck, C. S20-10
Brodbeck, U. S14-13, S14-14
Broger, I. S08-02
Bron, C. S06-26
Bronchti, G. S07-40
Brückner, B. S05-01
Bruderer, T. S14-21
Brugnera, E. S05-60
Bruinink, A. S07-33
Brun, R. S08-02
Brunat, W.L. S09-03
Brüne, B. S20-12
Brunner, A. S12-03
Brunner, D. S12-05
Brunschwig, K. S05-48, S05-52
Bryants, P.J. S03-26
Bub, A. S16-40
Buchala, A.J. S04-14, S04-17
Buchberg, A.M. S07-10
Buchegger, F. S03-24
Buchholz, C.J. S14-31
Buchstaller, A. S07-05
Buetti, E. S05-45
Bührer, A. S16-40
Bührer, S. S20-01
Bukauskas, F.F. S14-16, S14-17, S14-18
Bullough, P.A. S04-40
Burda, P. S10-05, S16-07
Burg, G. S03-02, S11-14
Burgat, J.M. S11-10
Burger, M.M. S06-20, S11-25
Burger, S. S12-12
Bürglin, T.R. S07-12
Burri, P.H. S13-11
Bütikofer, P. S14-13
Butz, S. S13-03

Callaerts, P. S12-06
Canavescini, S. S04-12
Canessa, C.M. S21-05, S21-07
Canevascini, G. S04-16, S05-46
Cao, G. S10-15
Capponi, A.M. S06-61
Car, B.D. S03-20, S06-13
Carafoli, E. S11-04, S11-16, S11-17, S11-18, S11-28, S14-07, S14-08, S14-09, S14-10, S14-11
Cardenas, M. S16-37
Cardinaud, R. S11-10
Cardinaux, J.-R. S06-42, S06-47
Cargnello, R. S09-04, S09-07
Carpenter, A.T.C. S05-31
Carrel, F. S16-11
Caskey, C.T. S07-10
Cassata, G. S06-46
Cathomen, T. S14-31
Catsicas, S. S07-41
Cattaneo, R. S14-31
Cavalli, F. S03-27, S16-35
Cavalli, V. S07-22
Cavin, C. S05-45
Cecchini, M. S06-07, S06-18
Celio, M.R. S09-03, S09-04, S11-02, S11-15, S11-27
Cerini, F. S08-09
Cerretelli, P. S01-04, S16-42
Cerutti, L. S20-09, S20-10

Cesura, A. S14-24, S17-21
Cesura, A.M. S17-25
Chanson, A. S04-15
Chapman, T. S12-11
Chaponnier, C. S13-06
Charnay, Y. S17-13
Chatton, J.-Y. S21-02
Cherif, D. S08-07
Chicaiza, G. S20-11
Choquard, P. S01-13
Chow, L.P. S04-28
Christ, B. S07-01
Christen, U. S03-23
Christina, N. S03-18
Church, D.J. S06-24, S06-27
Church, P. S18-08
Claassen, H. S01-03
Claeys, D. S21-04
Claret, F.X. S06-50
Clark, H. F. S12-08
Clarke, A. S03-36
Clarke, P.G.H. S07-38
Clarke, S. S17-14
Clausen, M. S04-26
Clever, B. S05-09
Cockell, M. S05-03
Cohen, D. S08-07
Cohen-Laroque, E. S16-42
Compassi, S. S21-15
Comte, M. S11-23
Conti, A. S08-06, S16-33, S16-34
Conti, M. S16-42
Conzelmann, A. S10-01
Coquoz, J.-L. S04-14
Corbett, M.S. S16-04
Corradin, G. S18-08
Corthésy, B. S14-20, S14-34, S16-28
Cosson, P. S10-03
Costello, E. S05-37
Cotecchia, S. S06-51, S14-03
Cottet, S. S14-20
Cotton, B. S12-11
Cousin, P. S05-44
Covacci, V. S16-34
Cox, J.A. S11-15, S11-22, S11-23
Crausaz, M. S04-20
Crespi, P. S04-37
Crestani, F. S03-34
Crompton, N.E.A. S20-14
Crottet, P. S16-13
Cueni, D. S08-03

D'Incalci, M. S16-35
Da Prada, M. S14-24, S17-21
Dainese, P. S14-11
Dalbey, R. S10-15
Dammann, R. S05-27
Danaie, P. S19-04
Darekar, P. S16-05
Daub, D.M. S17-23
Dauwalder, B. S12-11
Davies, K.E. S14-25
Davies, R.W. S17-27
Davis, W.C. S06-10
Davis-Daneshfar, A. S03-02
De Bilbao, F. S07-31
de Fraissinette, A. S16-14
De Grandi, P. S16-12
De Raad, S. S07-39
de Ribaupierre, F. S17-22, S01-10
De Rubertis, F. S05-58
de Tribolet, N. S17-23
Debanne, D. S02-04, S17-01
Déglon, N. S17-19, S17-20
Démollières, C. S10-03
Denton, R. S06-03
Denzer, A.J. S07-21, S07-22
Deplazes, P. S15-03
Descouts, P. S09-01
Deshusses, J. S06-60, S11-21
Desponds, C. S19-05
Di Paolo, G. S07-41
Dickinson, A. S02-10
Dickson, B. S12-05
Dietrich, D.R. S16-25, S16-26
Diggelmann, H. S16-30, S16-31
Dinter, A. S10-12
Diviani, D. S06-51
Djonov, V. S13-18
Do, K.Q. S17-11, S17-12
Dobbelaere, D. S06-14, S06-36
Döbbeling, U. S03-02
Docherty, A.J.P. S13-17
Doelz, R. S16-03
Doll, T. S07-09
Dominguez, M. S12-05
Donoho, G. S03-13
Douville, P. S05-63
Downward, J. S06-02
Drenhaus, U. S07-36
Dreyer, J.-L. S20-17
Droz, E. S09-01
Duan, H. S12-09
Dübendorfer, A. S12-13
Dubochet, J. S05-28
Dubois-Dauphin, M. S07-31
Dubremetz, J.F. S18-01
Duc, C. S21-05
Duc, T.M. S09-03
Dücker, K. S12-05
Duden, R. S10-03
Dudler, R. S04-25, S04-26
Dulloo, A.G. S16-18, S16-19
Dumermuth E. S10-07
Dummer, R. S03-02
Dunant, N. S06-33
Dunant, Y. S14-23
Durell, S.R. S14-04
Durussel, I. S11-15, S11-22

Edeline, J.M. S17-22
Eder-Colli, L. S07-34, S12-10, S14-23
Edgell, N. S06-03
Edwards, H.C. S11-05
Egelman, E.H. S05-30
Egerton, M. S10-04
Eggenberger, F. S16-03
Eggli, P. S11-27
Eggli, P.S. S07-37
Eichele, G. S07-10
Eichhorn, M. S06-36
El-Shemerly, M. S06-30
Elke, C. S06-22
Ellenrieder, C. S20-07
Emr, S.D. S10-03
Eng, V.M. S03-20
Engel, J. S14-33
Engelkampf, D. S11-24
Engels, M. S03-22
Eppenberger, H.M. S11-04, S13-03
Eppenberger-Eberhardt, M. S06-21, S11-04, S13-03
Erni, B. S14-22, S14-27
Ernst, S .A. S21-05
Escher, C.L. S04-10
Esposito, F. S18-08
Estade, M. S03-31
Estreicher, A. S17-23
Eugster, H.-P. S03-20, S11-08
Euringer, M. S07-06

Fahrni, J.F. S13-16
Faller, P. S03-07
Fankhauser, C. S20-09, S20-10
Fankhauser, H. S05-47
Farinelli, C.C.J. S16-19
Farkas, G. S05-59
Farmer, E.E. S04-01
Farthmann, B. S05-06
Fasciati, R. S16-26
Fasel, N. S05-44, S18-08, S19-05
Fast, V.G. S16-17
Fauchère, J.L. S06-57
Favre, D. S20-03
Federico, S. S11-21
Fedier, A. S13-04
Feindler-Boeckh, S. S13-09
Felix, G. S04-02
Felix, R. S06-07, S06-18
Felleisen, R. S18-06, S18-07
Felley-Bosco, E. S21-03
Feng, Z. S20-01, S20-11
Ferguson, M.A.J. S10-14
Ferralli, J. S07-09
Ferrazzi, P. S16-42
Filippi, B. S18-08
Fiocchi, R. S16-42
Fischbach, K.-F. S12-02
Fischer, Ch. S04-03
Fischer, G. S21-04
Fischer, M. S03-16
Fischer, N. S04-31
Fisher, R. S14-35
Fleck, O. S05-06
Fleisch, H. S06-07, S06-18
Fleischhauer, J.C. S16-39
Fleischmann, I. S12-11
Fleming, A.J. S04-12
Floth, C. S05-16
Flück, M. S06-32
Flynn, A. S06-03
Foletti, D. S14-07
Fontana, A. S17-24
Forrer, P. S20-08
Forster, I.C. S14-06
Foulstone, E. S06-03
Fraefel, C. S05-61
Fraichard, A. S04-15
Frank, G. S11-11
Frei, E. S12-09, S12-12
Frei, K. S16-26
Freiburghaus, A.U. S14-12
Frenk, E. S03-11
Frey, D.M. S07-26
Freydl, E. S04-32
Frieden, M. S06-58
Friis, R.R. S13-18, S20-01
Fritschy, J.M. S03-34, S17-10
Frixen, U. S13-19
Froguel, P. S08-07
Froster, U.G. S05-23
Früh, F. S03-19
Fu, W. S12-09
Fundel, S.M. S01-02
Fünfschilling, U. S07-08
Furlan, M. S13-08
Furrer, P. S05-28

Gabay, C. S03-31
Gabbiani, F. S13-06
Gabbiani, G. S13-06
Gähwiler, B.H. S02-04, S07-24, S17-01, S17-02
Galley, Y. S06-14, S06-36
Galli, C. S14-11
Galmiche, M. S03-24
Gander, J.C. S11-02
Garcia, I. S16-18
Gardet-Salvi, L. S04-28
Gärtner, T. S07-01
Gasser, D. S17-09
Gasser, M. S12-17
Gasser, S.M. S05-03, S05-42
Gassmann, M. S01-01, S01-05, S01-06, S01-07, S01-12, S03-13
Gatto, R. S19-08
Gausz, J. S05-59
Gavin, O. S04-15
Gaymard, E. S04-27
Gaynor, E.C. S10-03
Geering, K. S06-23, S10-08, S10-09
Gehr, P. S16-14, S16-43
Gehring, W.J. S05-55, S05-56, S12-06, S12-21, S12-22
Geiser, J. S01-13
Geiser, M. S16-43
Gendre, N. S12-07

Geng, Y. S05-33
Genini, M. S20-13
Georgiev, O. S05-14, S05-60, S05-62, S05-63
Gerber, B. S14-22
Gerber, H. S13-18
Gerster, T. S05-32
Gesemann, M. S07-21, S07-22
Gething, M.J. S14-28
Ghielmini, M. S16-35
Ghosh, R. S04-40
Giacobino, J.P. S06-11
Giger, R.J. S03-29, S07-06
Gilliéron, P. S21-18
Gilroy, C. S16-23
Giovannini, N. S03-21
Girardier, L. S16-18, S16-19
Glaser, T. S19-05
Goethals, M. S13-06
Goldschmidt-Clermont, M. S04-30
Gonzalez, C. S01-08
Goodman, C.S. S12-04, S12-08
Görlach, J. S04-07
Gosh, R. S04-41
Gotta, M. S05-03
Gottowik, J. S14-24, S17-21
Gottstein, B. S15-05, S18-02, S18-03, S18-04, S18-05, S18-06, S18-07
Götz, J. S03-17
Gotzos, V. S11-15
Gow, A. S07-35
Graber, R. S20-19
Graber, W. S07-37
Graf, H. S08-03
Graf, S. S04-25
Gramatikoff, K. S05-14
Grassi, B. S16-42
Greber, U. S03-04
Gremlich, S. S08-07
Grenningloh, G. S07-41
Greppin, H. S04-37
Grez, M. S03-05
Griesinger, C. S07-03
Grima, G. S17-11
Groner, B. S06-37
Groning, K. S19-07
Groppe, J. S12-22
Groscurth, P. S21-11
Gruber, M. S03-13
Grünberger, S. S04-01
Grüter, P. S19-07
Gschwind, M. S03-06
Gstaiger, M. S05-62
Guérineau, N.C. S17-01
Guerini, D. S11-04, S14-07, S14-08, S14-09, S14-11
Guern, J. S04-05
Guex, N. S04-08
Gugerli, P. S04-11
Guignard, J.P. S21-12, S21-18
Guillemin, K. S12-22
Guisan, B. S21-13, S21-14
Gundersen, D. S16-08
Günther, U. S03-34
Güntherodt, H.-J. S09-05
Gunz, D. S05-34
Gut, J. S03-23, S16-27
Gutknecht, R. S06-52
Gutteck, U. S17-12
Guy, H.R. S14-04
Gyurkovics, H. S05-59

Haab, P. S08-04
Haas, D. S04-21
Haas, F. S09-04
Hadjikhani, N. S17-14
Haenni, B. S13-11
Haerry, T. S05-56
Hafen, E. S12-05
Hagenbuch, B. S16-24
Hagenbüchle, O. S05-43
Hagens, G. S06-14, S06-36
Hagmann, M. S05-63
Hahlbrock, K. S04-04
Halasy, J. S06-18
Halder, G. S12-06
Halter, D. S12-01
Hamann, M. S03-32, S03-33
Hamburger, D. S10-04
Hamiche, A. S05-28
Hammer, M. S02-09
Hämmerle, S. S05-23
Hamou, M.F. S16-05
Hani, H. S08-07
Hanselmann, K. S16-25
Harder, B.A. S06-21
Hari, T. S14-13
Hartsuiker, E. S05-07
Hasler, D.W. S01-02
Hasler, R. S08-03
Hauptmann, G. S05-32
Hauri, H.-P. S10-10
Hauser, H. S21-15, S21-16
Hawken, A. S12-07
He, H. S12-14
Hecht, P. S03-22
Hegner, M. S09-05
Heider, H. S05-25, S06-45
Heierli, D. S04-38
Heimbeck, G. S05-31
Heimgartner, E. S05-24
Heizmann, C.W. S11-14, S11-24
Helenius, A. S03-04
Heller, N. S07-27
Hemphill, A. S18-02, S18-04
Henchoz, S. S05-58
Henderson, B. S19-03
Hennecke, S. S10-03
Hennig, P. S06-57
Hermann, B. S11-02
Hermes, W.T.J.M.C. S03-29
Herrera, P. S03-30
Herrmann, G. S06-10
Hersberger, M. S12-18
Hertel, C. S06-17, S06-19
Hertig, C. S13-03
Heyer, W.-D. S05-09, S05-11
Hihi, A.K. S06-50
Hilfiker-Kleiner, D. S12-13
Hill, F. S05-53
Hill, S.L. S02-07
Hiltbrand, E. S01-04
Hindges, R. S05-12
Hinz, U. S04-33, S04-34
Hirt, B. S05-37
Hirth, F. S12-04
Hoang-Van, K. S07-34, S08-09
Hobi, R. S05-19
Hochstrasser, D. S11-21
Hockfield, S. S17-28
Hoessli, D.C. S14-15
Hoffman, S.L. S18-08
Hoffmann, F. S05-38
Hoffmann-Pochon, N. S03-01
Hofstetter, W. S06-05, S06-07, S06-18
Hohl, D. S03-11
Hollerman, J.R. S02-01, S02-02
Holm, J. S14-29
Honegger, P. S07-25, S16-05
Honegger, U.E. S16-22
Hopkins, S.A. S16-12
Hoppeler, H. S01-03, S05-25, S06-45
Horisberger, J.-D. S21-07, S21-08
Hornung, J.P. S07-13
Hossle, J.P. S03-05, S06-08
Hottiger, M. S05-14, S05-15
Hovens, C. S05-62
Huang, Y.J. S05-49
Huarte, J. S03-30, S06-11
Huber, F. S14-27
Huber, G. S03-06
Huber, M. S03-11
Hübscher, U. S05-12, S05-13, S05-14, S05-15, S05-16
Huebner, S. S06-28
Hug, M. S06-44
Humbel, B.M. S13-23
Hummel, R. S06-25
Hummler, E S03-15, S07-02
Hunn, M. S06-26
Hunziker, E.B. S13-12
Hüttner, S. S06-08
Huwiler, A. S06-38

Ikai, A. S09-07
Iggo, R. S17-23
Ilangumaran, S. S14-15
Ilg, E. S11-14
Im Hof, V. S16-43
Imthurn, B. S05-04
Inoue, S. S16-09, S16-10
Inuoe, Y. S16-09, S16-10
Ireland, W. S16-23
Irigoyen, J.P. S13-19
Itin, C. S10-10
Ito, K. S12-01
Iwadate, H. S04-28
Iwata, T. S14-10, S14-11

Jabs, T. S04-04
Jäckli, P.A. S07-06
Jacob, R. S14-28
Jaggi, R. S06-46, S20-11
Jans, D.A. S06-28
Jans, P. S06-28
Jaquet, V. S07-35
Jarchow, J. S11-25
Jaton, J.-C. S07-34
Jaunin, P. S10-08
Jaussi, R. S05-24, S20-07, S20-08
Jeanguenat, N. S16-28
Jenck, F. S02-03, S08-01
Jentsch, S. S05-51
Jéquier, E. S08-05, S16-38
Joho, R.H. S14-05
Jones, R.S.G. S17-07
Jostarndt, K. S05-25, S06-45
Juillerat-Jeanneret, L. S16-05
Justice, R. W. S03-26

Kain, S.R. S16-06
Kalenga, B.M. S13-11
Kalousek, M.B. S06-39
Kalt, G. S11-19
Kamo, M. S04-28
Kampmeier, J. S18-01
Kanamori, A. S16-09, S16-10
Kappeler, F. S10-10
Karch, F. S05-59
Karrasch, S. S04-40
Karrer, U. S03-20
Kasas, S. S09-04, S09-07
Käser, L. S11-03
Kaufmann, H. S18-04
Kaufmann, J. S18-03
Ke, G. S20-01
Keegan, L. S05-56
Keller, B. S04-38
Keller, F. S16-41
Keller, H. S06-49
Keller, H.U. S13-04, S13-05
Keller, P.J. S05-04
Keller, W. S19-01, S19-02
Kemler, I. S17-24
Kemler, R. S13-03
Kernen, P. S09-05
Kessler, R. S06-39
Khoshbeen, M. S05-39
Kiefer, D. S10-15
Kim, Y.J. S16-13
Kindl, S. S07-03
Kinnon, C. S03-08
Kirby, K. S12-18
Kitajima, K. S16-09
Kitajima, X. S16-10
Kléber, A.G. S16-17, S16-39
Kleczkowska, H.E. S05-40, S05-41
Klemenz, R. S06-39

Kluge, I. S17-12
Kmecl, A. S04-26
Knecht, H. S03-03
Knoepfel, L. S05-62
Knoflach, F. S03-34
Knöfler, M. S05-43
Koch, D. S11-11
Koch, V. S16-27
Kohl, J. S16-37, S16-40
Köhler, G. S05-10
Kohler, M. S05-24
Köhler, P. S14-21
Kohli, J. S05-05, S05-06, S05-07
Koller, E. S21-17
Koller, E.A. S16-37, S16-40
Koller, T. S05-18, S05-27, S12-18
Komiyama, M. S13-14
König, M.F. S01-09
Köppel, R. S05-61
Kraehenbuhl, J.-P. S16-12, S16-28, S21-04
Kraev, A. S11-16, S14-10
Krähenbühl, St. S16-24
Krämer, A. S19-07, S19-08
Kramer, R. S06-38
Krämer, St. S10-06
Kramer, W. S05-06
Krapp, A. S05-43
Krasnow, M.A. S12-22
Krastanova, S. S04-11
Krause, R.M. S03-32
Krause, S. S19-01
Kretz, R. S17-16, S17-17
Kreuter, R. S01-12
Krishnan, S.N. S12-12
Krstic, R. S11-06, S17-03
Krummenacher, C. S16-30
Kubli, E. S12-11
Kucera, J.P. S16-16
Kucera, P. S01-10, S06-59
Kuenzle, C.C. S05-19
Kuhlemeier, C. S04-12, S04-13
Kuhn, A. S10-15
Kühn, C. S17-21
Kuhn, F. S20-16
Kühn, L. S19-03
Kuner, P. S06-19
Küng, M. S14-14
Küng, P. S06-34, S06-64
Kunz, D. S06-48
Kupferschmidt, H. S16-24
Kurz, H. S07-01
Kürz, L.L. S14-05
Kvietikova, I. S01-07

L'Hostis, C. S06-60
Laget, V. S12-24
Lahrtz, F. S17-24
Lambert, P.H. S06-57
Lämmle, B. S13-08
Landmann, L. S16-02
Lang, G. S14-24
Lang, U. S06-24, S06-27
Lang, Y. S03-34
Lankinen, M. S16-01
Larsson, B. S20-15
Lattion, A.L. S06-51
Lauber, M.E. S07-14
Lauener, R.P. S06-08
Lauper, N. S05-54
Laursen, N.B. S06-39
Lavenex, P. S02-06
Lavrijsen, S. S03-11
Lazzarini, R. S07-35
Leblanc, N. S04-05
Ledermann, B. S03-14
Lee, J.S. S13-19
Lee, L.L. S13-01
Lehmann, L. S16-39
Lei, X. S12-20
Leijendekker, W.J. S06-62
Letourneur, F. S10-03
Leuzinger, S. S12-04
Lezzi, M. S06-22
Li, J. S06-59
Li, W. S16-09
Li, Y. S14-21
Li, Z.-W. S03-17, S03-18
Lichtensteiger, W. S07-14, S16-21
Liechti-Gallati,S. S03-25
Lingner, J. S19-02
Lipke, P.N. S10-01
Lipp, H.-P. S03-18
Lipp, P. S11-26, S16-39
Liu, J.H. S03-32
Liu, Y. S14-05
Ljungberg, T. S02-01
Lo Russo, A. S06-55
Loepfe, E. S03-22
Lorenz, M. S06-13
Lorenzoni, P. S06-20
Losa, G.A. S20-19
Louvion J.-F. S06-53
Lovett, D.H. S13-01
Lucchesi, J.C. S12-13
Lucchini, R. S05-18, S05-26, S05-27
Ludin, B. S07-08
Luthi, F. S16-31

Maake, C. S08-02
Macaluso, E. S07-16
Macas, E. S05-04
Maestroni, G.J.M. S08-06, S16-33, S16-34
Maga, G. S05-13
Maggini, S. S16-15
Magistretti, P.J. S06-06, S06-42, S06-47, S17-05, S17-06, S17-07, S17-08, S17-09, S17-13
Magistris, M.R. S03-31
Magnenat, L. S05-50
Magyar, J.P. S07-04
Maier, P. S16-26
Malanga, M. S05-40
Malherbe, P. S14-24, S17-21, S17-25
Malina, H.Z. S11-07, S16-36
Mandriota, S.J. S13-20
Manova, A. S05-62
Mao, Q. S14-22
Marc-Martin, S. S04-11
Marconi, C. S16-42
Mari, M. S11-17
Marsden, K. S07-09
Marti, A. S20-11
Marti, H.H. S01-01, S01-05, S01-06, S01-07, S01-12
Marti, H.P. S13-01
Martin, C. S05-31
Martin, J.-L. S17-06, S17-08, S17-09
Martin, J.R. S02-03, S08-01
Martin, X.D. S11-07, S16-36
Martini, R. S03-10
Mary, T. S06-04
Marzorati, M. S16-42
Mathews, P.M. S10-09
Mathis, A. S15-03
Matile, H. S18-08
Mattei, M.-G. S11-24
Matthieu, J.-M. S07-35
Mattioni, T. S20-18
Matus, A. S07-08, S07-09
Mauch, F. S04-26
Maurer, F. S04-39
May, A. S21-06, S21-07
Maye, I. S16-14
Mayer-Jaekel, R.E. S13-09
McConville, M. S14-21
McKinney, R.A. S07-24, S17-01
Meda, P. S14-19
Medici, G. S16-41
Medilanski, J. S12-10, S14-23
Meier, P.J. S16-24
Meili, R. S20-06
Meiltz, A. S01-10
Meins, F.Jr. S04-24
Meise, M. S12-13
Melicharova, L. S20-14
Menotti, E. S19-03
Menoud, P.A. S05-39
Mermod, N. S06-43, S06-50
Messerli, J.M. S13-15
Messerschmitt, A. S06-29
Métraux, J.-P. S04-14, S04-17, S04-18, S04-19, S04-20, S04-24
Meuwly, P. S04-20, S04-24
Mey, J. S07-02
Meyer, C. S16-29
Meyer, U.A. S03-19
Mez, K. S16-25
Michalak, M. S11-01
Michel, C. S20-15
Michel, M. S13-12
Miescher, G.C. S06-16
Mignatti, P. S13-20
Minvielle-Sebastia, L. S19-02
Mirenowicz, J. S02-01
Mirkovitch, J. S05-38, S06-41
Möhler, H. S17-10, S17-26
Mölders, W. S04-20
Molinari, M. S11-17, S11-18
Monard, D. S16-32
Mondadori, C. S02-05
Monnat, J. S13-10
Monnet-Tschudi, F. S16-05
Montagne, J. S12-22
Montesano, R. S13-02, S13-20
Moore H.-P.H. S10-07
Mooser, F. S17-17
Moosmann, P. S05-22
Morabito, D. S06-61
Morabito, M.A. S17-28
Morano, I. S11-09
Moreau, J.-L. S02-03, S08-01
Morgenthaler, J-J. S16-11
Morris, M.A. S03-31
Mosig, D. S21-12, S21-18
Moss, S.E. S11-05
Mouginot, D. S17-02
Moule, K. S06-03
Mueller, M.E. S13-12
Mühl, H. S06-15, S20-12
Mühlebach, S.M. S07-15
Müller, A. S06-17
Müller, B. S11-14, S19-06
Müller, C.M. S07-03
Müller, F. S04-36, S05-48, S05-49, S05-50, S05-51, S05-52, S05-53, S05-54
Müller, H.P. S05-60
Müller, K.H. S05-60
Müller, L. S04-34
Müller, M. S03-20
Müller, M.E. S04-40
Müller, M.O. S04-09
Müller, N. S18-05
Müller, U. S03-17, S03-18
Münch, M. S05-04
Müntener, M. S11-03
Murata, J.I. S16-05
Murone, M. S06-54

Nabholz, M. S06-41
Naef, R. S14-30
Naegeli, H. S05-34, S05-35, S16-25
Nagamine, Y. S13-19
Nagy, F. S04-24
Naim, H.Y. S14-28
Nakamura, T. S13-02
Nardelli-Haefliger, D. S16-12
Nasir-ud-Din S14-15
Neely, M.D. S07-16
Nellen, D. S06-04
Nennstiel, D. S04-04
Ner, Sarbjit S. S12-01

Nesic, D. S19-07
Neuberg, D.H.-H. S07-04
Neuhaus, G. S04-03, S04-24
Neuhaus, J.M. S04-32
Nicholls, J.G. S07-16
Nichols, A. S03-30
Nicklas, W. S15-02
Niclou, S. S16-32
Nicod, P. S08-10
Nicolas, D. S11-06, S17-03
Niederer, P. S05-23
Niessen, M. S12-15
Nievergelt-Egido, M.C. S20-15
Niggli, E. S11-26, S16-39
Nilsen, T.W. S05-50
Nitsch, C. S11-19, S11-20
Nitsch, F.-M. S11-20
Noll, M. S03-26, S12-08, S12-09, S12-19, S12-20
Noll, T. S19-05
North, R.A. S14-01
Nöthiger, R. S12-13, S12-14, S12-15
Novier, A. S11-06, S17-03
Nürnberger, T. S04-04

O'Connell, M.A. S19-01
Oberson, J. S05-46
Obeso, A. S01-08
Ody, C. S06-11
Oellers, N. S12-05
Offord, E. S05-38
Offord, R.E. S08-09, S16-01
Oft, M. S13-22, S21-01
Oliver, B. S12-24
Olivier, R. S20-05
Olivieri, G. S06-16
Op den Camp, R. S04-13
Oppliger Leibundgut, E. S03-25
Orci, L. S03-30, S13-02
Orlando, V. S05-01
Osterheld-Haas, M.C. S07-13
Otten, U. S06-15
Otter, I. S20-02
Ozsahin, M. S20-14

Paganetti, P.A. S03-12
Pahud, G. S12-10
Palladini, F. S05-03
Palladino, T. S04-14
Pan, B. S14-08
Papanastasiou, P. S14-21
Paquot, N. S08-05
Pardo, B. S07-25
Pareek, S. S14-30
Paro, R. S05-01
Parsons, C.A. S05-30
Pasero, P. S05-42
Passaquin, A.C. S06-55, S06-62
Paul, K. S04-01
Pauletti, G.M. S10-06
Pauli, D. S05-57, S05-58, S12-23, S12-24
Pauls, T.L. S11-15, S11-22
Pawlowski, J. S13-16
Peitsch, M.C. S06-23
Peli, J. S13-22, S21-01
Pellegri, G. S06-42, S17-08
Pellegrinelli, N. S12-10
Pellerin, L. S06-06, S17-05, S17-06, S17-13
Peng, J. S16-23
Pennetta, G. S12-23
Pepper, M.S. S13-02, S13-20
Perotti, E. S04-15
Perriard, E. S13-14, S13-15, S16-20
Perriard, J.-C. S07-15, S13-14, S13-15, S16-20
Perrot-Rechenmann, C. S04-05
Perroud, M. S06-49
Perroud, P.F. S04-37
Petrova, T.V. S11-23
Pfeilschifter, J. S06-15, S06-25, S06-38, S06-48, S20-12
Philips, J.P. S12-18
Piani, A. S06-08
Picard, D. S06-53, S20-18
Piffareti-Yanez, A. S16-41
Pignat, W. S06-48
Pirrotta, V. S05-64
Pithon, E. S07-25
Pizer, L. S05-38
Pizzolato, G.P. S03-31
Pla, M. S06-41
Plasterk, R.H.A. S05-52
Plüss, K.M.A. S06-15
Pochon, N. S17-19
Podust, L.M. S05-16
Podust, V.N. S05-16
Poincelet, M. S14-15
Pollack, I. S12-03
Pollerberg, G.E. S07-05
Ponec, M. S03-11
Porret, A. S08-07
Porzig, H. S06-52
Potts, A. S16-12
Pountney, D.L. S01-02, S03-07
Pradier, J-M. S04-05
Pralong, E. S17-07
Preker, P. S19-02
Primi, M.-P. S07-38
Prod'hom, B. S06-59
Proud, C. S06-03
Prunell, A. S05-28
Puntschart, A. S05-25, S06-45
Puoti, A. S21-06, S21-07
Pytela, R. S16-08
Pythoud, C. S04-36

Qu, B. S16-09, S16-10
Quertain, F. S06-60
Quesniaux, V.F.S. S06-13
Quest, A.F.G. S06-26
Raddatz, E. S01-10, S01-11
Rader, C. S07-06
Radtke, F. S05-60
Raeber, A. S03-16
Raesecke, H.-R. S04-07
Rager, G. S07-36, S17-16, S17-17
Raggenbass, M. S07-31
Ramel, M.-H. S04-11
Ranscht, B. S21-17
Rastelli, L. S05-64
Ratcliffe, P.J. S01-14
Redmond, L. S17-28
Redpath, N. S06-03
Reichert, H. S12-04
Reichmann, E. S13-22, S16-29, S21-01
Reimmann, C. S04-21
Reinahrdt, E. S07-11
Reinecke, M. S08-02, S08-03
Reiner, O. S07-10
Reinhardt, C.-A. S07-26, S07-27
Reiss, G. S10-05, S16-07
Reize, C. S16-40
Resink, A. S17-26
Rettler, I. S03-11
Reuter, G. S05-58
Revelli, J.-P. S06-11
Reyes, G. S03-34
Reymond, C.D. S18-08
Reymond, P. S04-01
Richards, G. S17-21
Richter, F. S16-14
Richter, H. S04-08, S04-10
Ricken, A.M. S06-10
Rickert, C. S12-01
Riederer, B.M. S17-18
Riess, O. S16-04
Rietschin, L. S17-01
Riezman, H. S10-03, S10-04
Rigoni, P. S05-17
Rigual, R. S01-08
Rindisbacher, L. S03-24, S14-34, S16-28
Ringli, Ch. S04-38
Rivas, L.A. S01-05
Robert, Ph. S16-38
Roberts, M. S16-39
Robles, R. S13-08
Roch, F.A. S05-19
Roch-Ramel, F. S21-13, S21-14
Rochaix, J.-D. S04-29, S04-30, S04-31
Rochat, T. S03-35
Roduit, R. S08-08
Roggero, M.A. S18-08
Rohr, S. S16-16
Rolfs, A. S01-01
Romo, R. S02-01
Rose, K. S08-09, S16-01
Rosselli, M. S05-04
Rossier, B.C. S03-15, S10-09, S21-04, S21-05, S21-06, S21-07
Rossier, M.F. S06-61
Roth, J. S16-09, S16-10
Rothen-Rutishauser, B.M. S13-15
Rothenberger, S. S03-03
Roulet, E. S07-34, S14-23
Rousseau, M. S06-26
Ruberte, E. S06-04
Rudaz, C. S13-22, S16-29
Rudolph, C. S05-06
Rüegg, C. S16-08
Rüegg, M.A. S07-21, S07-22
Rüegg, U.T. S06-55, S06-62, S06-63
Rüfenacht, A. S04-22
Ruffieux, L. S04-09
Rülicke, T. S03-16, S03-17, S03-18
Rungger, D. S03-21
Rungger-Brändle, E. S07-32
Rusciano, D. S06-20
Rusconi, S. S03-05, S03-09, S05-10, S05-17, S05-22, S06-44
Russell, S.R.H. S05-31
Rusterholz, C. S06-41
Ruvkun, G. S07-12
Ryffel, B. S03-20, S06-13, S11-08

Sabovcik, R. S06-59
Sacks, W. S04-04
Sahin, A. S16-25
Sahli, R. S05-37
Salamin Michel, L. S05-29
Salem, N. S12-10
Sambrook, J. S14-28
Samori, B. S09-02
Sanchez, J.C. S11-21
Sanvito, F. S03-30
Sappino, A.P. S05-39, S13-21
Sappino, N. S05-39
Saraga, E. S21-04
Saraste, M. S06-01
Sartori, N. S05-29
Saurer, S. S20-01
Savioz, A. S17-27
Savoca, R. S07-07
Savoy, J. S08-04
Saydan, N. S05-24
Schachner, M. S14-02, S14-29
Schaefer, D.G. S04-35
Schäfer, B.W. S05-08, S11-14, S11-24, S20-13
Schaffner, W. S03-05, S05-14, S05-60, S05-62, S05-63
Schalet, A.P. S12-12
Schaller, A. S19-06
Schaller, D. S11-21
Schaub, M.C. S06-21, S11-09, S11-10, S11-11
Scheel, D. S04-04

Schein, C.H. S06-56, S07-26
Schenk, F. S02-06
Schick, B. S04-08
Schieferstein, U. S05-02, S05-21
Schild, L. S21-07
Schild-Poulter, C. S05-36
Schirlo, C. S16-40
Schläfli, O. S04-23
Schlumpf, M. S16-21
Schmid, J. S04-07
Schmid, V. S13-07
Schmidt, A. S07-17
Schmidt, I. S04-33
Schmidt, S. S20-09
Schmitt, I. S16-04
Schmuckli, J. S05-09
Schmutz, C. S14-32
Schneider, M. S04-14
Schneider, P. S10-14
Schneider, R. S07-29
Schneider, S. S03-05
Schneider, U. S14-31
Schneiter, Ph. S08-05
Schneiter, R. S12-15
Schneitz, K. S12-08
Schnell, L. S07-29
Schneuwly, S. S12-03
Schnyder, M. S03-20
Schödel, F. S16-12
Schorderet, D. S03-11
Schorderet, M. S07-39
Schrader-Fischer, G. S03-12
Schreyer, M. S16-08
Schrimpf, S. S03-29
Schröck, E. S06-16
Schulthess G. S21-15
Schultz, W. S02-01, S02-02
Schümperli, D. S20-16
Schunk, T. S14-22
Schürmann, P. S04-27, S04-28
Schutz, Y. S16-38
Schwab, M.E. S07-28, S07-29, S07-30
Schwalbe, P. S20-13
Schwaller, B. S11-02, S11-15, S11-26
Schweingruber, M.E. S05-47
Schweitzer, B. S07-04
Schweizer, M. S07-03
Schweizer, P. S04-17, S04-18
Schweizer, P.M. S05-20
Schwyzer, M. S05-61
Scott, M. S12-13
Scotti, A.L. S11-19, S11-20
Scuntaro, I. S16-22
Seger, R.A. S03-05, S06-08
Seghezzi, G. S13-20
Seiz Preiano, B. S14-09
Semenza, G. S09-05
Senften, M. S06-33
Serino, L. S04-21
Serra, A.L S16-43
Sessa, C. S16-35
Sétif, P. S04-31
Seum, C. S05-57
Seydoux, J. S16-18
Shaw, P. S05-44
Sidler, C. S07-33
Sidler, M. S04-25
Siegenthaler, P.A. S04-09
Siegrist, C.A. S06-08
Siles, M. S18-06
Simanis, V. S06-54, S20-09
Singh, A. S16-23
Singh, M. S05-61
Skutella, M. S06-63
Sleight, A. S02-03
Smith, A. S16-01
Sogo, J.M. S05-18, S05-26, S05-27
Sohrmann, M. S20-10
Soldati, D. S18-01
Soldati, G. S03-27, S16-35
Soldati, T. S13-14
Solletti, J.M. S09-03, S09-04, S09-07
Sommer, B. S06-05
Sommer, F. S09-03
Sommer, L. S05-43
Sonderegger, P. S03-29, S07-05, S07-06, S07-07
Soriani, M. S14-12
Soriano, J.V. S13-02
Spaans, M.C. S14-35
Spanel-Borowski, K. S06-10
Spaniol, P. S05-32
Spielmann, A. S04-11
Spierer, P. S05-57, S05-58
Spiess, M. S14-26
Spillmann, A.A.A. S07-30
Spindler, B. S21-10
Spörri, B. S06-09
Spring, J. S13-07
Spring, K.R. S21-02
Staab, S. S12-16
Stadelmann, B. S14-14
Stadler, H. S02-03
Stäger, S. S18-05
Stagljar, I. S10-05
Stampacchia, O. S04-29
Stancheva, I. S05-18
Staple, J. S07-41
Stark, G. S03-17
Stasiak, A. S05-30
Staubli, F. S11-13
Staudt, G. S06-38
Staufenbiel, M. S06-12
Stauffer, Y. S05-38
Stearns, S.C. S12-17
Steck, A.J. S06-16
Steinmann-Zwicky, M. S12-16
Stella, N. S06-06
Stieger, B. S16-24
Stocker, R.F. S12-07
Stöcklin, E. S06-37
Stöcklin, R. S08-09
Stone, S.R. S16-32
Störtkuhl, K.F. S12-07
Strasser, R.J. S04-41
Straumann, U. S01-05
Streit, P. S17-04
Stritt-Etter, A.L. S04-28
Strub, K. S03-28
Strutt, H. S05-01
Studer, D. S13-12
Studer, I. S07-33
Stutz, E. S04-39
Su, Q. S11-08
Suidan, H.S. S16-32
Suidgeest, P. S16-23
Summermatter, K. S04-19
Suter, D.M. S07-05
Suter, R. S16-21
Suter, U. S03-10, S07-04, S14-30
Swain, G.P S12-12
Szumiel, I. S05-41

Tabak, H.F. S10-02
Taborelli, M. S09-01
Tada, M. S17-23
Tair-Long, P. S13-07
Takagi, T. S11-23
Tallone, T. S05-10, S06-44
Tan, S.A. S03-01, S17-19
Tanaka, S. S05-02, S05-65
Tanner, I. S15-03
Tappy, L. S08-05
Tatout, C. S05-64
Taylor, C.R. S01-03
Taylor, V. S06-16
te Heesen, S. S10-05, S16-07
Technau, G.M. S12-01
Tempini, A. S01-13, S08-04
ten Heggeler-Bordier, B. S05-36
Tencalla, F.G. S16-25
Ternaux, J.P. S03-32
Testuz, J. S04-15
Thali, M. S10-13
Thalmeier, K. S06-52
Thanos, S. S07-02
Theler, J.-M. S14-15
Therianos, S. S12-04
Thoma, F. S05-02, S05-21, S05-65
Thomas, G. S06-65
Thomas, K. S07-36
Thomine, S. S04-05
Thompson, N. S08-10
Thompson, S.M. S02-04, S07-24
Thonney, M. S21-12, S21-18
Thorens, B. S08-07, S08-08
Tief, K. S07-17
Tilton, B. S20-16
Tinsley, J. S14-25
Tissot, M. S12-07
Tobler, H. S04-36, S05-48, S05-49, S05-50, S05-51, S05-52, S05-53, S05-54
Togni, G. S16-41
Togni, M. S16-34
Tomschy, A. S14-33
Torres-Schumann, S. S04-38
Tosic, M. S07-35
Touri, F. S17-18
Trachsel, H. S19-04
Trachsel, S. S13-05
Travers, A.A. S12-01
Tremblay, T. S02-02
Trewavas, A. S04-06
Trüb, T. S06-39
Tsacopoulos, M. S07-18
Tschanz, S.A. S13-11
Tsugita, A. S04-28
Turck, J. S13-01
Turian, G. S13-10
Tüscher, L. S01-03

Ungewickell, E. S09-05
Upton, A.L. S11-05
Urban, J. S12-01
Urich, M. S06-30
Utzig, S. S20-10

Vaistij, F.E. S04-30
Valenti, P. S05-64
Valiunas, V. S14-18
Vallélian, L. S04-18
Vallotton, M.B. S06-24, S06-27, S06-61
van den Bosch, H. S06-48
van der Straten, A. S12-05
Van Meir, E.G. S17-23
van Zutphen, L.F.M. S15-04
Vandekerckhove, J. S13-06
Vantini, G. S17-22
Varki, A. S16-13
Vasak, M. S01-02, S03-07
Vassalli, J.D. S03-30, S05-39, S13-20, S13-21
Vellani, F. S14-07
Verdumo, C. S03-15
Verhaagen, J. S03-29
Verrey, F. S21-09, S21-10, S21-11
Veuthey, A.-L. S07-18
Vickers, A. S14-35
Vilei, E.M. S11-04
Villa, A.E.P. S02-07, S17-22
Villa, M. S17-26
Vionnet, N. S08-07
Vogel, A.M. S05-32
Vogt, L. S03-29
Vogt, M. S05-25
Vögtli, M. S06-22
Volland, J.P. S06-57
Von Arx, P. S13-14
von der Weid, T. S06-43
von Fliedner, V. S16-08, S20-19
Vonderscher, J. S16-14
Voser, P. S08-04
Vu, L. S08-09

Wadley, Ch. S16-03
Waeber, G. S08-08, S08-10
Waelti, P. S02-08
Wagner, J. S13-12

Wagner, P. S09-05
Wahli, W. S05-36, S06-49, S06-50
Walker, G. S06-48
Walt, H. S05-23
Walter, P. S16-15
Walz, A. S06-40, S16-04
Wang, X. S21-08
Weber, E. S11-08
Weber, F.E. S21-16
Weber, J. S03-04, S11-03
Weber, R. S15-03
Webster, P. S03-04
Wehrle-Haller, B. S07-20
Weibel, E.R. S01-03, S01-09
Weingart, R. S14-16, S14-17, S14-18, S14-19
Weissmann, C. S03-16, S03-17, S03-18
Welker, E. S07-40, S17-18
Wellauer, P.K. S05-43
Wellinger, R. S05-02, S05-65
Wells, T.N.C. S09-01
Wels, W. S03-24, S06-37
Welsh, G. S06-03
Welzl, H. S02-08
Wenger, R.H. S01-01, S01-05, S01-07
Wenzel, A. S17-10
Werder, M. S21-16
Werlen, R.C. S16-01
Wermuth, B. S03-25
Wersig, C. S19-07
Weruaga Prieto, E. S11-02, S11-27
West, S.C. S05-30
Weston J.A. S07-20
Wetterwald, A. S06-05, S06-07, S06-18
Wey, E. S05-08
Wichmann,J. S02-03
Wicki, R. S11-24
Wicky, C. S05-54
Wickramasinghe, H.K. S09-06
Widmer, F. S04-08, S04-10
Widmer, H.-R. S01-03, S05-25, S06-45
Widmer, R.M. S12-18
Wiesmann, U.N. S06-09, S14-13
Wirz, T. S07-15
Wissler, M. S06-37
Wittek, R. S03-24, S14-32
Wittmann, C. S05-48, S05-52, S05-53
Wittmer, B. S19-04
Woerly, G. S03-20
Wohlwend, A. S03-30
Wolfer, D.P. S03-18
Wong, C. S07-10
Woods, D. F. S03-26
Wunderli-Allenspach, H. S10-06
Wyman, R.J. S12-12
Wymann, M.P. S06-35
Wyss, S. S10-13

Xiao, C-Y. S06-28
Xuelin, L. S12-20

Yamage, M. S18-03
Yamamoto, Y. S05-55
Yang, X. S19-08
Yano, K. S04-28
Yano, T. S01-04
Ye, Q. S11-12

Zanasco, J.-L. S04-29
Zapf, J. S08-02
Zenhausern, F. S09-06
Zerger, B. S04-29
Zetka, M., S05-54
Zhang, H.J. S14-05
Zhao, M. S11-08
Ziak, M. S16-09, S16-10
Ziegler, U. S03-29, S07-07
Ziemiecki, A. S06-31, S06-32, S06-34, S06-64
Zilian, O. S03-26
Zimmermann, S. S04-05
Zimmermann, V. S18-05
Zink, D. S05-01
Zinkernagel, R.M. S03-20, S15-01
Zryd, J.P. S04-33, S04-34, S04-35
Zuber, C. S16-09, S16-10
Zucca, E. S03-27
Zuellig, R.A. S14-25
Zufferey, R. S10-05, S16-07
Zühlke, R.D. S14-05
Zumsteg, V. S07-11
Zuo, X. S16-10
Zurbriggen, R. S20-17
Zürcher, G. S06-31, S06-32, S06-34, S06-64
Zürcher, H. S16-15
Zurich, M.G. S16-05
Zurlinden, A. S05-47
Zurn, A.D. S03-01, S17-19, S17-20
Zweifel, B. S05-35