## Gene Expression

S15-01

### REGULATION OF TRANSCRIPTION BY THE RETINOBLASTOMA TUMOUR SUPPRESSOR PROTEIN

Didier Trouche, Paul Lavender and Tony Kouzarides.
Wellcome/CRC Institute, University of Cambridge, Tennis Court Road, Cambridge CB2 1QR, UK.

The Retinoblastoma (RB) protein represses the activity of the S-phase-inducing transcription factors E2F1/DP1 by binding the activation domain of E2F1. We find that this E2F activation domain has structural and functional similarity to the N-terminal activation domain of E1A. Both activation domains have an identically spaced RB and CBP binding site. We show that CBP can act as a co-activator for E2F by binding and stimulating the E2F activation domain. Loss of CBP binding to E1A reduces E1A's ability to stimulate E2F activity. Our results suggest that the binding of both RB and CBP to E1A is required for E1A-stimulation of E2F.

We have identified a new target for RB. We present evidence that RB can repress the activity of a novel transcription factor, PBP1, isolated using a two hybrid screen in yeast. PBP1 has an HMG-box DNA binding domain and a transcriptional activation domain with an LXCXE RB-binding motif. This motif can bind RB *in vitro* and *in vivo*. Transfection experiments show that the LXCXE motif is essential for the activation capacity of PBP1. Binding of RB to this motif represses the activity of PBP1.

S15-02

### HERPES SIMPLEX VIRUS GENE EXPRESSION AND LATENCY

Chris M. Preston
MRC Virology Unit, Glasgow, Scotland, UK

Herpes simplex virus (HSV) is a large DNA containing virus which encodes at least 75 genes. Lytic infection of susceptible cells results in transcription of the genome in three temporal phases, immediate early (IE), early and late. Expression of the early and late genes is dependent upon the presence of IE proteins, which are known to be activators of transcription. The IE genes themselves are activated by the protein VP16 (= Vmw65, αTIF) a structural component of the virus which enters cells along with the genome during the early stages of infection. After primary infection of humans, HSV is retained lifelong in sensory neurons in a state known as latency. In some individuals, HSV periodically reactivates to form a "cold sore" blister. The control of gene expression during latency and reactivation is poorly understood at the molecular level. In latency, lytic genes are switched off but a single transcription unit encoding the latency-associated transcript (LAT) is active. I will discuss current ideas on the repression of lytic gene expression during latency, and will describe studies on the development of tissue culture systems for latency.

S15-03

### THE ROLE OF CYCLIN-CDK/E2F TRANSCRIPTION FACTOR COMPLEXES IN CELL CYCLE CONTROL

W. Krek[1], G. Xu[2], and D. M. Livingston[2]
[1]Friedrich Miescher Institut, Maulbeerstrasse 66, CH-4058 Basel
[2]Dana Farber Cancer Institute, 44 Binney Street, Boston, MA 02115

Temporal control of the transcription of genes whose products contribute to cell cycle advance is critical for normal cell cycle progression. Central to the temporal control mechanism is a relationship between the behavior of transcription factors, which contribute to cell cycle-dependent gene control, most notably E2F, and the activities of proteins which orchestrate individual cell cycle events, the cyclin-cdk complexes. The molecular mechanisms by which cyclin-cdk complexes modulate the activity of E2F and, in turn, E2F-dependent gene activity, is best understood in the case of E2F-1. According to a current model, activation of E2F-1 in late G1 is triggered by G1-specific cyclin-cdk complexes whereas its time-controlled inactivation in S results from stable complex formation with cyclin A-kinase which, in turn, eliminates E2F-1 DNA binding function. This form of time-dependent regulation provides one paradigm for how waves of gene expression are generated in mammalian cells at specific cell cycle intervals. Our recent results suggest that the timely elimination of E2F-1 DNA binding function by cyclin A-kinase is essential for orderly S phase progression and cell survival. Thus, one aspect of the largely unknown S phase regulatory function(s) of cyclin A-kinase may be mediated through its ability to modulate the behavior of E2F-1, and by extension, gene function.

S15-04

### CIRCADIAN GENE EXPRESSION AND THE PAR FAMILY OF bZIP TRANSCRIPTION FACTORS.

D. Lavery, E. Falvey, F. Fleury-Olela, P. Fonjallaz, L. Lopez Molina, L. Marcacci, V. Ossipow, and U. Schibler.
Department of Molecular Biology, University of Geneva, CH-1211 Geneva 4, Switzerland.

In mammals, circadian rhythms influence the expression of diverse genes in many different tissues. To understand the molecular mechanisms underlying such circadian gene regulation, we have focused on the PAR family of bZIP transcription factors, DBP, TEF, and HLF. In rodents, these highly similar family members display circadian rhythms in their mRNA and protein accumulation. Using biochemical and genetic approaches, we have begun to analyze the influence of PAR transcription factors on the expression of putative target genes involved in physiological systems including cholesterol homeostasis and sex hormone metabolism. Preliminary studies on mice homozygous for a disrupted allele of DBP suggest that functional redundancy, perhaps involving other PAR family members, may exist in the regulation of circadian target genes by DBP.

S15-05

### 3'-end cleavage and polyadenylation of eukaryotic messenger RNA precursors

S. Barabino, K. Beyer, D. Blank, A. Jenny, G. Martin, L. Minvielle-Sebastia, M. Ohnacker, P. Preker, U. Rüegsegger, Y. Strahm, T. Wiederkehr and W. Keller
Department of Cell Biology, Biozentrum, University of Basel, CH-4056 Basel, Switzerland.

The formation of the 3'-ends of nuclear pre-mRNAs leading to polyadenylated mRNAs proceeds in two main steps, endonucleolytic cleavage followed by polyadenylation. This simple reaction requires a surprisingly complex processing machinery. Purification and in vitro reconstitution of the cleavage and the polyadenylation reaction, as well as cDNA cloning and mutant analysis has allowed it to characterize many of the participating components and to establish their subunit composition. Cooperative interactions between the factors are important for processing complex assembly, RNA recognition, catalysis of poly(A) synthesis and the control of poly(A) tail length. Moreover, sequence comparisons of the mammalian 3'-end processing factors and their yeast homologs reveals basic similarities and important differences between lower eukaryotes and metazoans. A striking example of this are the poly(A) polymerases. These single-polypeptide enzymes have a modular sequence organization with different functional domains for primer binding, interaction with specificity factors, nuclear targeting and catalysis. Secondary structure prediction showed that their highly conserved catalytic core is similar to the palm domain of DNA polymerase β, the only protein of the family where the crystal structure is known. This homology extends as far as terminal deoxynucleotidyltransferase, cca: tRNA nucleotidyltransferase and streptomycin adenylyltransferase, an antibiotic resistance factor.

S15-06

### BOB1 SHOWS DIFFERENTIAL COACTIVATION OF OCTAMER PROMOTERS BY SELCTIVE TERNARY COMPLEX FORMATION WITH OCT FACTORS

Matthias Gstaiger, Oleg Georgiev, Lea Knoepfel and Walter Schaffner
Institute of Molecular Biology II, University of Zürich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland

We have previously shown that both octamer binding transcription factors namely the ubiquitous Oct-1 and the B-cell specific Oct-2A protein, can be enhanced in transcriptional activity by their association with the B-cell specific coactivator Bob1. Here we study the structural requirements for ternary complex formation and the coactivation function of Bob1. In analogy to DNA bound transcription factors, Bob1 has a modular structure consisting of an interaction domain (aa1-64) and a C-terminal domain (aa65-256), important for transcriptional activation. Mutational analysis has resolved a region of seven aa (aa 26-32) in the N-terminus of Bob1 essential for contacting the Oct-1 or Oct-2A POU-domain. In contrast to the viral coactivator VP16 (vmw65), that interacts with Oct-1 via the POU-homeodomain, Bob1 association with Oct factors requires residues located in the POU-specific domain (in collaboration with Hans van Leeuwen, Univ. of Utrecht). Since the same residues are also involved in DNA recognition we surmised that this association would affect the DNA binding specificity of the Oct/Bob1 complex compared to free Oct factors on octamer sequences. Although all of these octamer sites can bind free Oct-1 or Oct-2, ternary complex formation occurs only on a subset of octamer variants, leading to selective coactivation of octamer promoters. The results indicate a new level in selectivity that further our understanding in the regulation of the cell type specificity observed in octamer containing promoters.

S15-07

**Targeted disruption of the metal regulatory factor MTF-1 gene is lethal during mouse embryonic development**

Karl-Heinz Müller, Rainer Heuchel, Silvia Marino[1], Daniela Egli, Michel Aguet, Horst Blüthmann[2], Cagatay Günes, Hans-Peter Müller, Oleg Georgiev, Adriano Aguzzi[1] and Walter Schaffner
Molekularbiologie Universität Zürich, CH-8057 Zürich, Switzerland,
[1] Neuropathologie USZ, 8091 Zürich, [2] Hoffmann La-Roche PRTB, 4002 Basel

Upon crossing of mice heterozygous for MTF-1, no homozygous mutant offspring was observed pointing to an essential role of MTF-1 in embryogenesis. While null mutant embryos at 12.5 dpc were indistinguishable from wild type littermates, embryos at 14.5 dpc showed obvious liver degeneration with necrosis of the epithelial compartment, edema and congestion of the sinusoids. Already at 13.5 dpc, 60% of MTF-1 null mutant embryos showed incipient disruption of the regular epithelial net of the liver, but hepatocytes were still viable and showed a high rate of bromodeoxyuridine incorporation, suggestive of brisk proliferation activity. However, they expressed lower levels of cytokeratins. No living MTF-1 null mutant embryos older than 14.5 dpc were found. Amount and morphology of erythrocytes in knockout embryos are normal, thus we assume that haematopoiesis, which mainly takes place in the fetal liver at 13.5 dpc, is not affected. Since metallothionein I and II genes, the only known targets for MTF-1, are known not to be essential for mouse embryogenesis, we conclude that there exists at least one further target gene for MTF-1 which is essential for liver formation. The high similarity between MTF-1 and c-jun knockout phenotypes may mean that these genes affect the same developmental pathway.

S15-08

**CHROMATIN-MEDIATED TRANSCRIPTION REPRESSION AT YEAST TELOMERES,** Cockell,M., Palladino,F., Laroche,T., Gotta,M., and Gasser,S.M., Swiss Institute for Experimental Cancer Research, 1066 Epalinges, Switzerland

We have used a combined genetic, biochemical and immunofluoresence microscopy approach to study transcriptional repression of genes placed close to telomeres in yeast; The telomeric repeat-binding protein RAP1, the Silent Information Regulatory proteins Sir3 and Sir4 and histones H3 and H4 are all required for repression at telomeres and the clustered positioning of telomere-associated proteins at the nuclear periphery. Sir3 and Sir4 are thought to play a structural role in the formation of repressed chromatin, interacting with each other, with RAP1 and with the N-termini of histones H3 and H4 to form a multimeric complex. The degree of silencing is highly sensitive to both Sir3 and Sir4 dosage. However, overexpression results in increased or decreased repression respectively. We believe this may reflect differences between Sir3 and Sir4 in terms of interaction with other, as yet unidentified, components required for the structural and functional integrity of telomeres. We have carried out a two hybrid screen identifying proteins that interact with the Sir4 N-terminal domain. Interaction of two proteins is altered by mutagenesis of two $p34^{cdc2}$ kinase target sites. The roles of these proteins and the phosphorylation of Sir4 in telomeric repression are currently under study.

S15-09

**Krüppel Associated Box Domain as Transcriptional Repressor**

Peter Moosmann[1], Oleg Georgiev[1], Hans-Jürgen Thiesen[2], and Walter Schaffner[1]
[1] Insitut für Molekularbiologie der Universität Zürich, Abteilung II, Winterthurerstrasse 190, CH-8057 Zürich
[2] Institut für Immunologie der Universität Rostock, Schillingallee 70, D-18055 Rostock, Germany

The Krüppel associated box (KRAB) domain, consists of about 65 aa, is present in around one third of the estimated 300-500 Krüppel type zinc finger proteins, and is subdivided into a mandatory A box (~45aa) and an optional B box (~20aa) (1). The KRAB domain is conserved at least in humans, mice and the clawed toad *Xenopus* (2). The physiological role of this domain is unknown, and so are the target promoters of the KRAB containing zinc finger factors (2,3). When fused to a DNA binding domain, the KRAB domain represses efficiently transcription, regardless of the orientation and the location of the respective DNA recognition site (our results and 3). In yeast, the KRAB domain cannot repress transcription. In a yeast two hybrid screen we have isolated several clones that interact with the KRAB domain of Kox-1, including one that contains a characteristic ring finger subtype . Currently, we are analysing these clones.

1. Bellefroid et al., Proc. Natl. Acad. Sci. USA 88 (1991), 3608-3612
2. Margolin et al., Proc. Natl. Acad. Sci. USA 91 (1994), 4509-4513
3. Pengue et al., Nucl. Acids. Research (1994) 22, 2908-2814

S15-10

**Progesterone receptor form A as a transcriptional Repressor of form B and glucocorticoid receptor**
Barbara Huse and Sandro Rusconi, *Institut de Biochimie, Université de Fribourg, 1700 Fribourg*
The human progesterone receptor (PR) exists in two distinct isoforms, form A and form B. PR-A differs from PR-B in missing 164 amino acid in the N terminus. PR-B functions as a transcriptional activator of progesterone responsive genes, while PR-A is mostly inactive in most cells. Interestingly in some cell and promoter contexts PR-A acts as a trans-dominant repressor of PR-B. Other groups have shown that the PR-A mediated repression is not restricted to PR but includes glucocorticoid receptor (GR) and estrogen receptor (ER). Furthermore, it was suggested that PR-A can effectively inhibit the other steroid receptors even when expressed in a sub-stoichiometrical amount. We could only partially confirm these observations. Our results indicate that (A) the capacity of ER repression is very minimal and cannot be distinguished from squelching; (B) the PR-A has only minor effects when expressed at sub-stoichiometrical levels. Since our earlier studies indicated that complete truncation of the PR N terminus does not produce dominant negative PR, we postulated that a repression domain must be contained in the region between position 165 and 538. To better narrow down of this modulator domain we made progressive deletion mutants and chimeras that demonstrates that this domain is confined within 120 amino acid in the N terminus region and is not movable to other receptors.

S15-11

**THYROID HORMONE RECEPTORS: BINDING AND TRANSACTIVATION PROPERTIES**
JEANNIN E., DESVERGNE B., Institut de Biologie Animale, CH-1015 Lausanne, Switzerland.

Thyroid hormone receptors (TR) are nuclear receptors that bind diverse thyroid hormone response elements (TRE). Natural TRE identified so far in wild type promoter can exhibit a palindromic (Pal), an inverted palindromic (IP) or a direct repeat (DR) orientation of the two hexameric half-sites. TR preferentially bind to these elements as heterodimer with RXR. However, they are also able to bind as homodimer and monomer. The role of these various complexes observed in vitro in the T3 response remains to be clarified. We have analysed and compared the binding and the transactivation properties of two TR isoforms, TRα1 and TRβ1. The experiments were performed with two structurally different TRE, a DR4 and an IP6 found in the malic enzyme (ME) and in the myelin basic protein (MBP) promoter, respectively. We observed differential transactivation potencies of TRα1 and TRβ1 depending on the promoter studied. The most striking feature is a poor activation of the MBP promoter by TRα1 while TRβ1 is still able to efficiently mediate the T3 response. This can be correlate to the formation of a strong monomer binding pattern of TRα1 to the IP6 while it is absent when using TRβ1 or when tested on the DR4. To further our understanding of the specific role of monomer in vivo, we performed mutation either in the TRE and/or in the TR.

S15-12

EARLY ALDOSTERONE REGULATED mRNAs IN A6 KIDNEY CELLS
Spindler, B., Mastroberardino, L. and Verrey, F., Physiologisches Institut der Universität Zürich, CH-8057 Zürich
Adrenal steroids produce after a lag period of approximately 45min an increase in the transcellular sodium reabsorption across A6 epithelia. This effect is thought to be mediated by the action of transcriptionally regulated gene products on Na channels and Na pumps. To identify potential mediators we used a modification of the differential display reverse transcription PCR method. We compared approximately 5000 bands corresponding to mRNAs from two sets of control and aldosterone (40min/1h) treated epithelia. To reduce the number of false positives we considered as regulated only bands showing a roughly 2-fold or larger change in intensity for both sets of RNAs. According to these criteria 0.6% of the bands were up- and 0.1% downregulated. We have cloned 4 of these cDNA fragments corresponding to early adrenal steroid upregulated RNAs (ASUR). The regulation was confirmed by Northern blotting. After 1h aldosterone treatment the ASUR1 signal was increased 5-fold, the ASUR2 over 10-fold and the ASUR4 1.5-fold. The signal for ASUR2 is disappearing after 6h aldosterone treatment, whereas those of ASUR1 and ASUR4 remain high after 16h aldosterone. No significant similarities of the cDNA fragments to known nucleotide sequences were observed using the BLAST algorithm. We are now screening *Xenopus laevis* libraries to obtain larger cDNA clones. To functionally test the potential role of ASURs in Na channel and/or Na pump regulation we are establishing a system for regulated expression of exogenous proteins in A6 epithelia.

S15-13

**CLONING AND CHARACTERIZATION OF THE MOUSE *EXO* GENE ENCODING AN RNA TURNOVER EXONUCLEASE**

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

*S.cerevisiae* Xrn1 (=Sep1, Kem1, Rar5, Dst2, Stpb, Skil) is a multi-functional protein with roles in RNA metabolism, microtubule function and possibly in telomere metabolism. To get insight into the *in vivo* role of Xrn1 we cloned its mammalian homolog - the mouse *exo* gene. The full length cDNA was assembled and used for functional studies in yeast. The mouse cDNA was able to complement the slow growth phenotype, the sporulation defect and the benomyl hyper-sensitivity as well as an RNA turnover defect in the processing of the 35S rRNA precursor in a *xrn1Δ S.cerevisiae* strain. Western blotting analysis of protein isolated from mouse tissues, using antibodies against EXOp, confirmed the presence of a protein the size predicted by DNA sequence analysis. The mouse cDNA was overexpressed in yeast and the 200 kDa EXOp was purified by chromatography. *In vitro* studies showed the protein to posess 5'-3' exonuclease activity on ssDNA and RNA. EXOp was found to have high specificity for binding and cleavage of G4 tetraplex DNA and RNA. Taking together our data show that mouse *exo* is the structural and functional homolog of *S.cerevisiae XRN1* and *S.pombe Exo2*.

S15-14

DETECTION BY SINGLE CELL RT-PCR OF mRNAs ENCODING ALTERNATIVELY SPLICED FORMS OF THE PACAP/VIP TYPE 1 RECEPTOR (PVR1) IN IDENTIFIED RAT GONADOTROPHS.

Rawlings, S.R., Bépoldin, L., Journot, L.*, & Schlegel, W. *F.R.M., University of Geneva, and *C.N.R.S.-U.P.R. 9023, Montpellier, France.*

Pituitary adenylate cyclase-activating polypeptide (PACAP) and vasoactive intestinal polypeptide (VIP) regulate the activity of a variety of anterior pituitary cell types. However the receptor subtypes mediating these effects are not known. In the present study, freshly dissociated, indo-1-loaded, anterior pituitary cells were plated onto coverslips, and the cytosol of individual gonadotrophs (identified by their $Ca^{2+}$ response to LHRH) was collected using an adaptation of the patch clamp technique. The use of the reverse transcription-polymerase chain reaction (RT-PCR) with PVR1-specific primers revealed the expression of two alternatively spliced forms of this receptor in such cells. In 9 gonadotrophs tested in this way, 1 cell expressed only the short form of the receptor (PVR1*s*), 6 cells expressed only the "long" form (PVR1*hop*), and 2 cells expressed both receptor forms. Since the splice variants occur in the third cytosolic loop, a region important for receptor-G-protein coupling, such differential PVR1 expression may have important consequences for the regulation of intracellular signalling, and luteinising hormone synthesis and release, by PACAP in individual gonadotrophs.

S15-15

**ROLE OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS IN SKIN LIPOGENESIS AND DIFFERENTIATION**

L. Michalik, S. Beck, L. Martin, W. Wahli and H. Keller
Institut de Biologie animale, Université de Lausanne, 1015 Lausanne

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors of the superfamily of nuclear hormone receptors. They play an important role in lipid metabolism; in response to peroxisome proliferators and natural fatty acids, they regulate gene transcription of several enzymes involved in hepatic and adipose tissue fatty acid metabolism. The aim of this project is to elucidate a role of PPARs in skin lipogenesis and differentiation. The outermost layer of the epidermis, the stratum corneum, forms the physical barrier of the skin. It is composed of intercellular lipid lamellae and terminally differentiated, dead keratinocytes (corneocytes) that are filled with a keratin-matrix complex surrounded by the cornified envelope. Consistent with a proposed role of PPARs in epidermal gene regulation, we found that PPARα and β are expressed in the epidermis, but not dermis, of mouse embryos, using in situ hybridization. PPARβ is first detected at day E11, whereas PPARα is only seen from E13 on. Furthermore, PPARα is expressed in all cell layers of the epidermis, whereas PPARβ is only expressed in the basal layer. Both PPARs are also expressed in hair follicles as soon as they start to form. To study the function of PPARs in skin homeostasis and differentiation, we are analyzing the effects of PPAR activators on mouse skin in organ cultures. Preliminary results show a thickening of the epidermis, suggesting that activation of PPARs leads to hyperkeratosis.

S15-16

CYTOCHROME P450 (P450) INDUCTION BY PHENOBARBITAL

K. Hoffmann, F. W. Früh and U. A. Meyer, Dept. of Pharmacology, Biozentrum, University of Basel, CH-4056 Basel

The mechanism by which Phenobarbital (PB) and related substances induce P450 mRNAs is still unclear. The effects observed in vivo are lost in all established hepatoma or related cell lines due to the dedifferentiation of these cells. Primary hepatocyte cultures and cell lines which spontaneously differentiate in culture therefore offer a promising system for the investigation of the mechanism *in vitro*.

We have used a 4.8kb fragment derived from the 5' flanking sequences of the chicken CYP450 2H1 gene to investigate the putative mechanism of inducibilty by PB in reporter gene assays. Various parts of this fragment were recombined separately to generate a mini-gene highly inducible with PB.

In parallel a human cell line (Caco2-Tc7) which differentiates in cell culture was observed to show P450 inducibility by PB as demonstrated by quantitative PCR and Differential Display techniques.

The combination of both systems is presently used to lead to a better understanding of the mechanism of PB-induction in the liver.

S15-17

**OVER 50 GENES REGULATED BY PHENOBARBITAL IN CHICKEN EMBRYO LIVER**

Felix W. Früh, Klaus Hoffmann and Urs A. Meyer; Department of Pharmacology, Biocenter of the University, CH-4056 Basel

Phenobarbital (PB) elicits pleiotropic effects in the liver including induction of enzymes involved in xenobiotic metabolism. To what extent PB also influences the expression of other genes and if these genes show similar features, implicating a distinct mechanism of cellular response to PB is not known. To investigate the spectrum of genes regulated by PB, mRNA differential display was adapted to chick embryo and chick embryo hepatocyte cultures and used to screen approximately half of the mRNA population of chick embryo liver tissue *in vivo*. Twentynine cDNA fragments regulated by PB (6mg/egg for 48h) were identified. 18 fragments were upregulated and 11 fragments were downregulated. Twenty strongly regulated fragments were selected for further analysis. Nucleotide sequencing revealed 3 types of genes: (1) genes already known to be induced by PB like GST and UDP-GT, (2) genes not previously reported to be regulated by PB (fibrinogen, complement factor H, retinal glutamine synthetase, EF1δ, apoB) and (3) several new genes with so far unknown functions. Extrapolated to the entire mRNA population of this system, the results described here indicate that more than 50 genes are differentially expressed after PB treatment. In addition to induction, PB also represses a considerable number of genes.

S15-18

IN VITRO TESTING OF RECOMBINANT RAT LITHOSTATHINE — A PUTATIVE CALCIUM CARBONATE CRYSTAL INHIBITOR

D. Bimmler, R. Graf, Y. Choffat#, G.A. Scheele*, T.W. Frick
Dept. Chirurgie, Universitätsspital, Zürich. #) Zool. Institut Univ. Zürich. *) Beth Israel Hosp., Harvard Med. School, Boston.

Pancreatic juice is supersaturated with respect to calcium carbonate. The hypothesis that an inhibitor of calcium carbonate crystal formation is secreted into pancreatic juice was supported by the identification of lithostathine, a 16 kDa protein that displays calcium carbonate crystal inhibitor activity *in vitro*. So far, only native purified lithostathine has been tested at concentrations between 1 and 6 µM. To validate this hypothesis with protein which is free of pancreatic contaminants, we have recently produced recombinant rat lithostathine through baculovirus driven expression. We have now purified the recombinant rat lithostathine and tested its crystal inhibitor activity *in vitro*: it exhibited an activity equal to the one reported for native lithostathine. When lithostathine was digested with trypsin to yield a fragment shortened by 11 amino acids, it completely lost its inhibitory activity, probably due to a transition from a globular to a thread-like protein. The chemically synthesized N-terminal undecamer exhibited no inhibitory activity, either. However, when globular control proteins such as trypsinogen, human serum albumin and soybean trypsin inhibitor were tested at similar concentrations, they were as active as lithostathine. We therefore doubt a) that lithostathine's crystal inhibitor activity is specific, and b) that - in the micromolar range - the assays used to demonstrate this activity are valid tests to assess crystal inhibition *in vivo*.

S15-19

**INTRODUCTION OF FAMILIAL ALZHEIMER'S DISEASE MUTATIONS IN MOUSE EMBRYONIC STEM CELLS BY HOMOLOGOUS RECOMBINATION.**

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

Several pathogenic mutations have been found in families with early onset Alzheimer's disease (AD), most of them located on the ß-APP gene framing the coding region for Aß, the major protein component of senile plaques in AD. Homologous recombination was used to introduce some of these mutations in mouse ES cells by two different approaches. The "hit and run" procedure leads to an intermediate duplication of the genomic region induced by an insertion vector carrying pathogenic mutations. In a second step elimination of this duplication by intrachromosomal recombination results in either reversion to wt or mutant genotype. The second approach includes two subsequent homologous recombinations induced by two independent replacement vectors. Selection genes introduced at the desired locus are eliminated in a second replacement event with homologous DNA carrying familial AD mutations. Although the second approach allows the introduction of multiple mutations the "hit and run" procedure appears to be more efficient.

S15-20

DIFFERENTIAL UTILZATION OF TRANSFECTED ACTINS IN CULTURED RAT ARTERIAL SMOOTH MUSCLE CELLS

Nicole Mounier and Giulio Gabbiani. Université Lyon-1, F-69622 Villeurbanne, and Department of Pathology, CH-1211 Geneva 4

The distribution of isoactins within cytoskeletal structures was studied in cultured smooth muscle cells (SMCs) by transfecting the corresponding genes. These genes carry an epitope tag discriminating the tagged actin from endogenous actins (kindly provided by Pr J. C. Perriard, E.T.H., Zürich). Over-expression of a supernumerary actin in transient assays was not lethal for SMCs. α-cardiac, and α- and γ-smooth muscle actins were preferentially recruited into well organized stress fibers and there was a good correlation between tagged actin and F-actin localization. In contrast, transfected cytoplasmic β- and γ-actins accumulated in granular structures scattered within the cytoplasm or in large deposits at the cell periphery, and rarely into filaments. In general SMCs transfected by cytoplasmic actins showed less stress fibers than untransfected cells. We conclude that when present in excess, isoactins are differentially sorted by SMCs. Muscle actins are preferentially recruited into stress fibers while cytoplasmic actins disturb the microfilament network. These results strengthen the concept of a functional diversity of the different actins.

S15-21

**FUNCTIONAL ANALYSIS OF MYOMESIN IN ES CELL DERIVED CARDIOMYOCYTES: ISOLATION OF THE MOUSE MYOMESIN GENE**

I.U. Haussmann , S. Keller and J.-C. Perriard
Institute for Cell Biology, ETH-Hönggerberg, CH-8093 Zürich

Myomesin is a 185kD myofibrillar protein which is located in the middle of the sarcomer, in the so called M-band. It seems to play an essential role for correct assembly of myofibrils, possibly by anchoring the titin filament system to the thick myosin filaments. Myomesin has no counterpart in smooth or non-muscle cells and we want to study its involvement in myofibrillar architecture by disrupting the endogenous myomesin gene in embryonic stem (ES) cells.
Therefore, we have screened a genomic mouse AB-1 library with a 5' cDNA probe of rat myomesin. The resulting clones were analysed by Southern blot analysis, PCR and sequencing. A comparison with known cDNA sequences has shown that three clones contain the exon, which encodes the putative myosin binding domain of the myomesin protein. The myomesin gene will be inactivated by homologous recombination replacing this exon with a neomycin resistance gene. The effects on cardiac cytoarchitecture will be investigated in ES cell derived cardiomyocytes, obtained from embryoid bodies.

S15-22

**MAPPING OF FUNCTIONAL DOMAINS OF MYOMESIN IN LIVING RAT CARDIOMYOCYTES**

D. Auerbach, S. Bantle, M. Leu and J. C. Perriard
Institute for Cell Biology, ETH Hönggerberg, CH-8093 Zürich

Myomesin is a 185 kD structural protein exclusively present in the M-band of cross-striated muscle. Possible binding partners within the M-band include myosin and the giant protein titin.
In order to investigate the myofibril binding activity present in myomesin we generated several contructs consisting of either full-length myomesin or deletion mutants containing different domains labelled with an epitope tag derived from the vesicular stomatitis virus for easy discrimination against endogenous myomesin. In addition to the full-length cDNA, constructs encoding either single N-terminal domains or fragments consisting of several such domains were transfected into neonatal rat cardiomyocytes. The full-length construct and all mutants which included domain 2 (immunoglobulin-like domain) showed a clear cross-striation similar to the staining pattern of endogenous myomesin, whereas a construct encoding domain 3 (immunoglobulin-like domain) alone showed a diffuse staining. This leads to the conclusion that the myofibril binding activity present in the N-terminal portion of myomesin must be located within domain 2 of the molecule.

S15-23

**ESSENTIAL ROLE FOR TRANSCRIPTION FACTOR AP-2 IN CRANIOFACIAL DEVELOPMENT**

**H. Schorle*, P. Meier+, M. Buchert+, R. Jaenisch*, and P.J. Mitchell+. *Whitehead Institute for Biomedical Research, Nine Cambridge Center, Cambridge, MA 02142-1479 and Dept. of Biology, MIT, Cambridge, MA 02138, USA, and +Institute for Molecular Biology, University of Zürich, 8057 Zürich.**

**Transcription factor AP-2 has been implicated as a cell-type-specific regulator of gene expression during vertebrate embryogenesis based on its expression pattern in neural crest cells, ectoderm, and the nervous system in mouse and frog embryos. AP-2 is prominently expressed in cranial neural crest cells, a population of cells that migrate from the lateral margins of the brain plate during closure of the neural tube in 8 - 9 dpc mouse embryos. Inductive interactions between cranial neural crest cells and neighboring cell types are required for the formation of many structures in the vertebrate head. In order to study the role of AP-2 during development, we have undertaken a targeted mutagenesis of the AP-2 gene in the mouse. Homozygous AP-2 mutant mice died at birth with cranio-abdominoschisis, full facial clefting, and defects in cranial ganglia and sensory organs. The primary defect, failure of the cranial neural folds to close at 9 dpc, coincided with increased cell death in subsets of cranial neural crest cells and brain plate neuroepithelial cells. The AP-2 mutation reveals a fundamental requirement for this regulatory protein in craniofacial development.**

S15-24

**PERIPHERAL MYELIN PROTEIN 22 BELONGS TO AN EXTENDED GENE FAMILY**

V. Taylor, A. A. Welcher* and U. Suter
*Institute of Cell Biology, ETH-Hönggerberg, 8093 Zürich,*
**Amgen Inc. Thousand Oaks CA-91320, USA*

Peripheral myelin protein 22 (PMP22) is expressed in many tissues but mainly by Schwann cells as a component of compact myelin of the PNS. Mutations affecting PMP22 are associated with hereditary motor and sensory neuropathies. Although these phenotypes are restricted to the PNS, PMP22 is thought to play a dual role in myelin formation and in cell proliferation. We have cloned and characterized epithelial membrane protein-1 (EMP-1), a putative four-transmembrane protein of 160 amino acids with 40% identity to PMP22. EMP-1 and PMP22 are structurally related and co-expressed in most tissues but with differences in relative expression levels. EMP-1 is most prominently expressed in the gastrointestinal tract, skin, lung and brain but not in liver. EMP-1 protein can be detected in specific gastric epithelial cells in a pattern consistent with plasmamembrane association. Furthermore, EMP-1 and PMP22 mRNA levels are inversely regulated in the degenerating rat sciatic nerve after injury and by growth arrest in NIH3T3 fibroblasts. The discovery of EMP-1 as the second member of a novel gene family led to the identification of the lens-specific membrane protein 20 (MP20) as a third but distant relative. The proteins of this family are likely to serve similar functions possibly related to cell proliferation and differentiation in a variety of cell types.

S15-25

**VIP, PACAP AND NORADRENALINE INDUCE THE TRANSCRIPTION FACTORS C/EBPβ AND C/EBPδ IN MOUSE CORTICAL ASTROCYTES: INVOLVEMENT IN cAMP-REGULATED GLYCOGEN METABOLISM.**

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

We have previously described in astrocytes a transcription-dependent induction of glycogen resynthesis by the vasoactive intestinal peptide (VIP) or noradrenaline (NA), which is mediated by cAMP. Since the CCAAT/enhancer binding protein (C/EBP) family of transcription factors is implicated in the regulation of energy balance in hepatocytes and adipocytes, we performed Northern and Western blot analyses and observed an induction of the C/EBPβ and -δ isoforms by VIP, PACAP or NA. A pharmacological analysis indicated that the induction by NA is mediated by β-subtype adrenergic receptors. VIP, PACAP and NA therefore probably increase C/EBPβ and -δ expression via the cAMP second-messenger pathway. This was further confirmed by the induction of both proteins by 8-(4Br)-cAMP or forskolin. Induction of C/EBPβ and -δ mRNA by VIP occurs in the presence of a protein synthesis inhibitor. Thus, *c/ebpβ* and *c/ebpδ* can be considered as cAMP-inducible immediate-early genes in mouse astrocytes. Moreover, transfection of astrocytes with expression vectors producing the transcriptionally active form of C/EBPβ, termed LAP, or C/EBPδ enhance the glycogen resynthesis elicited by NA, whereas an expression vector selectively producing the inactive form of C/EBPβ, termed LIP, reduces this resynthesis. These results suggest that C/EBP isoforms regulate energy metabolism-related enzymes in astrocytes.

S15-26

SCREENING FOR BRAIN-SPECIFIC cDNAs ENCODING PURINE SEQUENCE-BINDING PROTEINS USING A YEAST ONE-HYBRID ASSAY

Robert Hellwig and Bernhard Lüscher, Institute of Pharmacology, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland

Analysis of the 5' flanking region of the $GABA_A$ receptor δ subunit gene revealed a novel tandemly repeated purine sequence element that is recognized by several ubiquitous and tissue specific DNA-binding proteins. One of these factors, brain-specific factor 1 (BSF1) was only observed in protein extracts from brain. The regional distribution in brain and the temporal induction during differentiation of primary neurons closely matched the expression pattern determined for the δ subunit mRNA, suggesting that BSF1 could play a role in differential transcription of the corresponding gene (Motejlek et al., 1994, JBC 269, 15265-15273).

To screen for cDNAs encoding BSF1, a yeast one hybrid screen was designed. HIS3 and a lacZ reporter genes containing various distinct purine-rich sequence elements within their 5'-flanking region were constructed and transformed into yeast to engineer strains suitable for screening of brain-derived cDNA libraries. Interestingly, several of the purine elements inserted into the reporter gene resulted in significant constitutive activation of the reporter genes in the absence of exogenous activators, suggesting that purine-specific DNA-binding transcription factors that activate the reporters are present in yeast. Eventually, constructs suitable for screening were identified that showed only minimal activation in the absence of cotransfected cDNAs. Screening of a mouse cerebellum cDNA library for histidine auxotrophy resulted in 60 colonies. Thirteen out of nineteen of these isolates resulted in significant activation of the lacZ reporter gene. An initial characterization of these cDNAs will be presented.

S15-27

**CLONING AND FUNCTIONAL EXPRESSION OF A METALLOENDOPEPTIDASE FROM HUMAN BRAIN WITH THE ABILITY TO CLEAVE ß-APP SUBSTRATE PEPTIDE**

**Annick Thompson, Gerda Huber and Pari Malherbe**
**Pharma Division, Preclinical Research, F. Hoffmann-La Roche Ltd, CH-4002, Basel, Switzerland**

Using a combination of PCR and hybridisation screening, we have isolated a cDNA clone for a metalloendopeptidase (h-MP78) from a human temporal cortex library. This 2.5 Kb cDNA encodes a 689-amino acid protein with a predicted molecular mass of ca. 78.5 kDa. The primary structure of h-MP78 exhibits high similarity to those of porcine (94%) and rat (92%) thimet oligopeptidase. Expression of the cDNA in HEK-293 resulted in the production of an active enzyme able to cleave a chromogenic ß-APP derived substrate peptide KTEESEVKM-P-nitro-anilide. RNA blot analysis of various human tissues revealed one major species of h-MP78 mRNA of 2.55 kb. The highest level of mRNA was found in the brain.

S15-28

HUMAN MEPRIN, A TRANSMEMBRANE ZN-METALLOENDO-PEPTIDASE, IS EXPRESSED IN VILLUS ENTEROCYTES BUT NOT IN CRYPT CELLS IN HUMAN SMALL INTESTINE

D. Lottaz, U. Luginbühl and E. Sterchi
Institute of Biochemistry and Molecular Biology
*University of Berne, Switzerland*

Human Meprin (EC 3.4.24.18) is a heterodimeric Zn-metalloendopeptidase expressed in epithelial cells of human small intestine. On the basis of a characteristic protease domain, Meprin has been included in the Astacin family of Zn-metal-loproteases (Dumermuth et al), some of them being involved in embryonal development and tissue differentiation. We investigated the possible role of Meprin in the regulation of enterocyte differentiation by localizing the enzyme in crypt cells versus villus cells by in situ hybridisation and immunohistochemistry on human small intestinal tissue sections: Meprinα and Meprinβ specific mRNA was detected in crypt cells *and* villus enterocytes, in contrast to the protein itself, which appears only in terminally differentiated villus cells.

S15-29

**S100 PROTEINS ARE TRANSCRIPTIONALLY REGULATED BY DIFFERENTIAL DNA METHYLATION IN HUMAN TUMORS**

Roland Wicki, Beat W. Schäfer and Claus W. Heizmann
Abteilung für Klinische Chemie, Kinderspital, Universität Zürich, Steinwiesstrasse 75, CH-8032 Zürich

Calcium-binding S100 proteins became of major interest because of their differential expression in tumors. At least ten S100 genes were found to be clustered on human chromosome 1q21, a region frequently rearranged in many tumors. S100A4 and S100A2, both located on the cluster, are oppositely regulated. S100A4 is thought to play an important role in metastasis and S100A2 may play a role in suppressing tumor cell growth since it is found in normal but not in transformed cells. To examine the regulation of the two genes, we determined the DNA methylation states of both promotors in a normal and a tumor cell line and found significant differences. We showed that the grade of DNA methylation correlates perfectly with the expression pattern of S100A2 and S100A4 and that DNA methylation might be a mechanism controlling the different expression of S100 genes in tumor cells.

S15-30

**CONSTRUCTION AND CHARACTERIZATION OF A CARTILAGE-SPECIFIC cDNA LIBRARY**

D. Belluoccio and B. Trueb, MEM Institut für Biomechanik, Universität Bern, 3010 Bern

Differentiated chondrocytes secrete an extra-cellular matrix composed of collagens (types II, IX, XI), proteoglycans (aggrecan) and noncolla-genous proteins (CMP, COMP). In rheumatoid arthritis and osteoarthrosis, a dramatic loss of cartilage is observed. This loss is known to be due to the degradation and altered expression of extracellular matrix molecules.

In order to identify novel cartilage-specific components we are currently constructing a subtracted cDNA library. Chick cDNA molecules prepared from sternal chondrocytes are hybridized to biotin-labeled cDNA molecules obtained from skin fibroblasts. Hybrids formed between the two cDNA populations are removed using streptavidin. Chondrocyte-specific unlabeled cDNA is amplified by the polymerase chain reaction. By applying this method, we expect to find, in addition to already known sequences, novel genes that are transcriptionally active in chondrocytes.

S15-31

**IN *SITU* STROMAL EXPRESSION OF UROKINASE IN COLORECTAL ADENOMAS CORRELATES WITH EPITHELIAL DYSPLASIA**

I. Sordat, P.Chaubert*, P. Protiva°, J. Benhattar*, E. Saraga*, L. Guillou*,L. Mazzucchelli*, C. Trân-Thang, A. L. Blum°, G. Dorta°, B. Sordat. ISREC, Epalinges,°Division of Gastroenterology CHUV/PMU Lausanne, Institutes of Pathology *Lausanne and *Bern.

An increase in urokinase-type plasminogen activator (uPA) and a decrease in tissue-type plasminogen activator (tPA) are associated with the transition from normal colorectal mucosa to adenomas. *Methods*: using serial sections from 25 adenomas, plasminogen activator related caseinolytic activity was determined by in *situ* zymography, blocking selectively uPA or tPA. uPA, tPA and type 1 plasminogen activator inhibitor (PAI-1) mRNA distribution was investigated by non radioactive in *situ* hybridization. Low and high grade dysplasia was mapped histologically on consecutive H&E stained sections. *Results*: 23 of 25 adenomas showed predominantly uPA-related caseinolytic activity located in the periphery whereas tPA-related activity was mainly in central areas of adenomas. 15 of 25 adenomas expressed uPA mRNA located in clusters of fibroblast-like stromal cells. Foci of uPA mRNA expressing cells coincided with areas of uPA activity. The probability to find uPA mRNA is significantly higher in areas with high dysplasia. Cellular PAI-1 mRNA was stromal and did not correlate with the grade of dysplasia. No tPA mRNA signal could be detected beyond the noise level. *Conclusions*: these results show that an upregulation of uPA is associated with foci of highly dysplastic epithelial cells. They suggest that epithelial-stromal interactions promote uPA-mediated extracellular proteolysis in adenomas.

S15-32

DIFFERENTIAL EXPRESSION OF mRNA's IN RAT KIDNEY CORTEX INDUCED BY LOW PHOSPHATE-DIET.

Custer M., Murer H. and Biber J., Institute of Physiology, University Zürich, 8057 Zürich.

The adaptive response of proximal tubular cells to a low Pi-diet is generally characterized by an increase of apical Na/Pi-cotransport. Part of this adaptive phenomenon is independent of hormonal factors and therefore represents an intrinsic property of the proximal epithelial cell. The mechanisms leading to this adaptive response are largely unknown. To obtain more insight into the adaptive response to a low Pi-diet we have used the method of differential display PCR to identify gene products regulated by a low Pi-diet. Total RNA was isolated from kidney cortex of rats which have been fed for three days either with a high (1.2% Pi, controls) or with a low (0.1% Pi) Pi-diet. After reverse transcription using oligodT-8 primers PCR was performed using a set of arbitrary 13-mers. Analysis of approximately 1000 bands revealed that a low Pi-diet induces two kinds of changes; 20 bands were found to be increased and 10 bands were found to be decreased. By Northern blot analysis using poly(A)$^+$RNA isolated from kidney cortex of control and adapted rats changes as observed by DD-PCR of six bands could be confirmed. Identified gene products have been subcloned and sequenced. Further analysis of these gene may yield novel insights into the mechanisms involved in the intrinsic adaptive response of proximal tubular cells to low $P_i$.

S15-33

**GENERATION OF HIGH AFFINITY RABBIT ANTIBODY FRAGMENTS BY REPERTOIRE CLONING**

**M. Foti, A. Beliën, P. Ricciardi-Castagnoli, M. Ackermann and M. Suter**
**University of Zurich, Switzerland, University of Milan, Italy**

Rabbit monoclonal antibodies (RmAb) are not routinely obtained by eukaryotic cell fusion techniques. Therefore, mAb production by repertoire cloning was evaluated. The immunoglobulin repertoire in rabbit is generated by using a restricted number of VH or VL for VDJ or VJ rearrangement, respectively. This greatly simplifies the amplification of Ig genes by PCR and unbiased expression by prokaryotes.

Libraries were generated by cloning of PCR amplified VH-CH1 and VL-CL fragments from RNA of immunised rabbits into the pComb3 phagemid vector and expressed on filamentous bacteriophage. The utility of the libraries was assessed by selection of phage bound to solid phase attached antigen. Antigen specific clones thus obtained and appropriate controls were further analysed by ELISA. The RmAb Fab fragments were shown to be antigen specific. In order to facilitate purification of these Fab fragments, the corresponding genes were subcloned into the pJuFo vector. This vector was engineered to contain the sequence encoding six consecutive histidine residues and the gene encoding the E. coli alkaline phosphatase. The antibody fragments were thus purified on metal chelate affinity chromatography and the affinity constant was determined using the grating coupler sensor indicating affinities of up to 6,1 x $10^{-8}$. The data therefore indicate the feasibility of generating high affinity rabbit antibody by repertoire cloning.

S15-34

A GENE FUSION EXPRESSION SYSTEM FOR DELIVERY OF THE OPEN READING FRAME 3 (ORF3) OF PORCINE EPIDEMIC DIARRHOEA VIRUS (PEDV) INTO EUKARYOTIC CELLS

A. Schmitz, M. Suter, and M. Ackermann
Institute of Virology, Faculty of Veterinary Medicine, University of Zürich, Winterthurerstrasse 266a, CH-8057 Zürich, Switzerland

The function of ORF3 of the Coronavirus PEDV is unknown, the corresponding gene product has not yet been identified, and specific antibodies could not be generated. Preliminary data suggest that ORF3 or its gene product may represent a virulence factor of the virus.

To test this hypothesis, we have cloned ORF3 into the pJuFo phagemid cloning system (Gene, 137: 69) which allows the co-expression of four recombinant proteins linked to each other by the Jun and Fos leucine zippers. In our construct, ORF3 was cloned 5' of Fos and fused to the *E.coli* heat-labile enterotoxin subunit B (LTB) which was inserted 5' of Jun. LTB is known to bind to the ganglioside GM1 receptor facilitating internalization into eukaryotic cells. In future experiments, LTB will (i) serve as the sole adjuvant to obtain specific antibodies to ORF3 by immunization, (ii) allow to monitor antibodies to the ORF3-LTB fusion protein bound to GM1 receptor coated ELISA plates and (iii) be used to study *in vitro* and *in vivo* the fate of eukaryotic cells which have been delivered with ORF3-LTB.

S15-35

XANTHURENIC ACID MAY AFFECTED CHAPERONE ACTIVITY OF α-CRYSTALLIN

H.Z. Malina and X.D. Martin, Laboratory of Experimental Ophthalmology, c/o Swiss Institute for Experimental Cancer Research; CH-1066 Epalinges/Lausanne, Switzerland.

An activity of kynurenine aminotransferase (KAT) was observed in the cortex of cataractous lenses but not in the cortex of young lenses.KAT activity transformed 3-hydroxykynurenine into xanthurenic acid. In the presence of xanthurenic acid, the fluorescent complex of α- and β-crystallin lost its fluorescence when heated at 55 °C, for one hour. In the absence of xanthurenic acid, this complex lost only ten percent of its fluorescence under these conditions. The heat protection of lactate dehydrogenase activity was observed in the presence of α-crystallin, but this effect was diminushed when the α-crystallin was conjugated with xanthurenic acid. The accumulation of xanthurenic acid in human lenses with aging, and its ability to form covalent conjugates with α-crystallin may be involved in the observed decrease of its chaperone activity in human lens with aging.

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S15-36

PROMOTER REGION OF THE MURINE AND OK-CELL NPT II

C. M. Hartmann, H. Hilfiker and H. Murer
Institute of Physiology, Zurich

The $Na^+$/Pi-cotransporter type II is a regulated protein and expressed only in proximal tubular cells of the renal cortex. The related cDNA has been cloned from several species.The murine NPT-2 gene is organized into 13 exons and 12 introns and has a size of ~16kb. In order to study the mechanisms underlying tissue specific expression as well as regulation at the transcriptional level, we have cloned the promoter region of the murine (mNPT2) and OK-cell (oNPT2) type II transporter gene. Putative CAAT and TATA boxes were found in the two genes. For the murine NPT-2 gene two transcriptional start sites (at positions -9 and -10 when compared with the mouse cDNA) were identified by primer extension and 5'RACE. A 484 bp (mNPT-2) and a 327 bp (oNPT-2) 5' flanking fragment comprising CAAT box, TATA box and a part of exon I, was cloned in front of a luciferase reportergene. The constructs were able to drive the luciferase gene expression when transiently expressed in the OK-cell line.

S15-37

**A POLYOMA-BASED EPISOMAL VECTOR IN MOUSE EMBRYONIC STEM CELLS AND DIFFERENTIATED RODENT CELL LINES**

Camenisch[1] G., Gruber[2] M., Wenger[1] R.H., Donoho[3] G., Berg[3] P. and Gassmann[1] M.
[1]Inst. of Physiology, University of Zürich-Irchel, CH-8057 Zürich, [2]Inst. of Cellbiology, ETH, CH-8093 Zürich. [3]Department of Biochemistry, Beckmann Center, Stanford University Medical School, Stanford, CA 94305-5425

We characterized a polyoma virus-based plasmid vector that is episomally maintained in mouse embryonic stem (ES) cells without altering the pluripotent status and the capacity to populate an embryo. The plasmid vector pMGD20*neo* contains the polyoma origin of replication harboring a mutant enhancer (PyF101), a modified gene encoding the large-T protein and the neomycin (*neo*) gene. The plasmid replicates in ES cells and is maintained as an extrachromosomal element in 15% of G418-resistant clones. Cells that maintain the autonomously replicating plasmid can efficiently replicate and maintain a second supertransfected plasmid that carries the polyoma origin of replication. (*PNAS*, **92**, 1292-1296, 1995)
Several murine and a rat cell line were transfected either with pMGD20*neo* or with pMGD20*neo*-derived constructs harboring two different fragments of the *hprt* gene. Both vectors were able to replicate efficiently in ES cells, mouse embryonic carcinoma (F9) cells, mouse adenocarcinoma (RAG) cells, mouse erythroleukemia (MEL) cells, and mouse L-fibroblasts (L929), but were not detected in rat aortic endothelium carcinoma (RAEC) cells. Furthermore, circular pMGD20*neo*-based and *hprt*-containing vectors used to target the endogenous *hprt* gene in ES cells showed no enhancement of homologous recombination compared to their linear counterparts.

S15-38

**ATF-1 AND CREB-1 BIND CONSTITUTIVELY TO THE HYPOXIA-INDUCIBLE FACTOR-1 (HIF-1) DNA RECOGNITION SITE**

*Kvietikova I., Wenger RH., Marti HH., and Gassmann M.,*
*Institute of Physiology, University of Zürich-Irchel, CH-8057 Zürich,*

The hypoxia-inducible factor-1 (HIF-1) was first described as an essential factor for hypoxia-inducible erythropoietin (EPO) gene transcription. HIF-1 activity has also been found in cell lines which do not express EPO, suggesting that HIF-1 is part of a widespread oxygen-sensing mechanism. In EMSA's, HIF-1 DNA binding activity is only detectable in nuclear extracts of cells cultivated in a low oxygen atmosphere. In addition to HIF-1, a constitutive DNA binding activity also specifically binds the HIF-1 probe. CRE and AP-1 oligonucleotides efficiently competed for binding of the HIF-1 probe to this constitutive factor, whereas the HIF-1 activity itself remained unaffected. Monoclonal antibodies raised against the CRE binding factors ATF-1 and CREB-1 supershifted the constitutive factor while *jun* and *fos* family members, which constitute the AP-1 factor, were immunologically undetectable. Moreover, recombinant ATF-1 and CREB-1 bound HIF1 probes either as homodimers or as heterodimers, suggesting a new binding specificity for ATF-1/CREB-1 (NAR, **23**, 1995, in press). As these transcription factors are targets for protein kinase A-dependent phosphorylation, our findings suggest that the cAMP-signalling pathway might be involved in oxygen-sensing.

S15-39

**OXYGEN-DEPENDENT GENE EXPRESSION AND ERYTHROPOIESIS IN EMBRYOID BODIES.**

*Bichet S., Wenger R., Marti H.H., Bauer C., Gassmann M., Physiol. Inst., Universität Zürich, Winterthurerstr. 190, CH-8057 Zürich.*

We studied the response to reduced oxygenation during the differentiation of embryoid bodies (EBs). To determine the EBs' capability to sense oxygen tension and to specifically respond to low ambiant oxygen by modulating gene expression we quantified aldolase A and vascular endothelial growth factor (VEGF) mRNA since expression of both genes is upregulated by hypoxia in a variety of cells. Compared to the normoxic controls, we found increased aldolase A and VEGF mRNA levels after exposing 8-9 days old EBs to 1% oxygen. We also analysed the regulation of erythropoietin (Epo), the main regulator of erythropoiesis which is known to be regulated in an oxygen-dependent manner. Nine days old EBs increased their Epo mRNA level when exposed to hypoxia. Since Epo stimulates the maturation of erythrocytes and in turn leads to an increase in hemoglobin, we studied the regulation of hemoglobin synthesis in EBs. Interestingly, we detected 2 peaks of hemoglobin synthesis at day 6-7 and 15-16, probably mimicking the *in vivo* situation where the embryonic globin, present from day 5 p.c. precedes the adult globin, which progressively substitutes the embryonic globin from day 10 p.c. on. Furthermore, preliminary results showed that a 24h hypoxic incubation of 7 days old EBs results in an up to 1.6 fold increase of hemoglobin. Since Epo mRNA is present as early as in ES cells, we are currently investigating the role of Epo during erythropoiesis within EBs. In summary, we speculate that hypoxia could represent a physiological stimulus during development.

S15-40

**CYTOCHROME B558 NADPH OXIDASE IS NOT THE OXYGEN SENSOR**

Marti, H.H.; Schuerer-Maly, C.C.; Kvietikova, I.; Bauer, C.; Gassmann, M.; Maly, F.E. and Wenger, R.H.
Institute of Physiology, University of Zürich, 8057 Zürich

Reduced oxygenation of a variety of cells results in transcriptional upregulation of several genes, including erythropoietin, VEGF and glycolytic enzymes. Recently, the heme protein cytochrome b558 of the NADPH oxidase complex has been proposed as a key component of the oxygen sensing mechanism. Mutations in the subunits of cytochrome b558 result in Chronic Granulomatous Disease (cytb$^-$ CGD), an inherited disorder in humans characterized by reduced microbicidal activity due to deficient superoxide generation. To test whether NADPH oxidase is involved in oxygen sensing, we exposed wild type B cell lines as well as cytb$^-$ CGD-derived B cell lines, deficient in either p22$^{phox}$ or gp91$^{phox}$ subunit, to hypoxia (1% oxygen) or $CoCl_2$ (100 mmol/L) and compared the mRNA levels of VEGF and aldolase with the untreated controls. We found unimpaired basal and inducible expression of VEGF and aldolase mRNA in all four cytb$^-$ CGD-derived B cell lines compared to wild type cells. Furthermore, reconstitution of cytochrome b558 expression in cytb$^-$ CGD-derived B cells by transfection with p22$^{phox}$ or gp91$^{phox}$ expression vectors did not modify VEGF and aldolase mRNA expression. Thus, cytochrome b558 of the NADPH oxidase complex appears not to be essential for hypoxia-activated gene expression. (Blood 87, (1996), in press).

S15-41

**Hypoxia, a Novel Inducer of Acute Phase Gene Expression in a Human Hepatoma Cell Line**

Rolfs A., Marti H.H., Bauer C., Gassmann M. and Wenger R.; Physiol. Inst. Universität Zürich-Irchel, CH-8057 Zürich

A widely used model for studying transcriptional regulation of hypoxic induced gene expression is the hepatoma cell line HepG2. To identify additional genes expressed during hypoxia, we differentially screened a cDNA library derived from hypoxic (1% $O_2$) HepG2 cells. Two genes could be identified, one encoding aldolase A, and the second one encoding $\alpha_1$-antitrypsin, a member of the acute phase (AP) responsive proteins. AP proteins are liver-derived plasma proteins whose production during inflammation is either up- ([+]AP reactants) or down-regulated ([-]AP reactants). In the present study, we demonstrate that on the mRNA level hypoxic stimulation of HepG2 cells led to (i) an induction of the [+]AP reactants $\alpha_1$-antitrypsin, $\alpha_1$-antichymotrypsin, haptoglobin, complement C3, and $\alpha_1$-acid glycoprotein; (ii) a down-regulation of the [-]AP reactant albumin; (iii) an up-regulation of the [-]AP reactant transferrin; and (iv) unchanged levels of the [+]AP reactants $\alpha$- and $\beta$-fibrinogen as well as hemopexin. Cycloheximide inhibited hypoxic up-regulation of AP mRNAs demonstrating that *de novo* protein synthesis is required. Nuclear run-on assays indicate that the hypoxic increase in AP mRNAs is mainly due to transcriptional regulation. The hypoxic response was compared to interleukin-6 stimulated AP, the results suggest that the response to hypoxia overlaps with, but is not identical to, the AP response mediated by interleukin 6. (J. Biol. Chem. 270, 46 Nov 17, 1995) Regulatory sequences leading to hypoxic AP induction are under current investigation.

S15-42

**Post-transcriptional regulation of expression of XId in *Xenopus* oocytes and embryos.**

Afouda B. A., Zhang H., Reynaud S. and Spohr G.
Department of Cell biology, University of Geneva.

Determination and differentiation of various developmental systems depend on transcription factors of the bHLH family. These factors are negatively regulated by HLH proteins such Id, which lack the basic DNA binding domain. In *Xenopus* , Id is expressed at a low level in oocytes and at a high level after MBT. We have identified a putative regulatory element on the 3'-untranslated part of XId mRNA. In order to be able to determine the role of this sequence, we have constructed chimeric genes which contain different part of the XId trailer attached downstream to the luciferin coding sequence. The activity of the *in vitro* transcribe mRNAs have been determined by microinjection into oocytes and fertilized eggs. We find that a palindromic sequence located between 22 and 37 nucleotides upstream from the poly-adenylation site stimulates XId translation.

S15-43

**MOLECULAR ANALYSIS OF PRONEPHRIC DEVELOPMENT IN *XENOPUS LAEVIS*: CLONING OF PAX-2 CDNAS**

Nicole Heller & André W. Brändli
Institute of Cell Biology, ETH-Hönggerberg, CH-8093 Zürich

Organogenesis of the vertebrate kidney is initiated by induction of the pronephros. The molecular aspects of this process are poorly understood. Recently, it has become apparent that genes of the Pax family of transcription factors appear to control the formation of various organs in the embryo. In particular, Pax-2 is required for proper development of the kidney in mice and man [Epstein (1995) Proc. Natl. Acad. Sci. 92: 8566-8573].
We have screened a *Xenopus* stage 28 cDNA library and isolated cDNA clones for two *Xenopus* Pax-2 genes, XPax-2a and XPax-2b. These cDNAs are most likely derived from two separate, pseudoallelic Pax-2 genes. XPax-2a and XPax-2b encode proteins of 393 aa and 462 aa, respectively. It is likely that the size differences are caused by alternative splicing. We are currently conducting studies on the developmental expression pattern of the *Xenopus* Pax-2 genes by Northern blot analysis and *in situ* hybridization. Further, we are planning to use ectopic expression to assess whether Pax-2 might function as an early control gene for kidney organogenesis in vertebrates.

S15-44

**ID EXPRESSION DURING *XENOPUS* DEVELOPMENT**

Reynaud, S., Zhang, H., Fisher, J. and Spohr, G.
Department of Cell Biology, University of Geneva

The myogenic regulatory factors (MRF) form a group of transcription factors whose biology describes a paradigm in which genetic switches control the fate of an entire cell lineage. The MRFs are negatively regulated by ID HLH proteins which lack a basic DNA binding domain. Id factors inhibit MRF activity by acting in a dominant negative manner. In order to determine the role of Id during development we have isolated and characterized the Id genes expressed in *Xenopus*. In order to study the functional role of XId during early embryogenesis, we have used an overexpression approach. Overexpression of XId leads to a reduction of muscle formation. As a strategy for identifying cis-regulatory elements we have constructed chimeric genes consisting of different 5' deleted promoter elements attached to the bacterial chloramphenicol acetyl tranferase encoding sequences. The activity of these chimeric genes was determined by microinjection into embryos. We have defined a 200 bp region that is necessary for the correct temporal regulation of the gene.

S15-45

**Analysis of gene expression in the zebrafish embryo**

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

In an attempt to isolate new genes that are developmentally regulated during early zebrafish development, we are conducting a large-scale screen using differential display reverse transcription-PCR (DDRT-PCR): DDRT-PCR is performed with RNA from various stages between fertilization and early organogenesis. Amplificates that show temporally differential expression are subsequently tested for tissue specific expression by whole mount *in situ* hybridization. Results from this ongoing screen will be presented. In the course of pilot experiments, two differential band amplificates have been found that show tissue-specific expression. One is expressed in the entire forming mesoderm, the other is expressed in the tip of the head process and later in the hatching gland. The corresponding genes are currently being characterized in more detail.

Procedures for the effective and reliable production of transgenic fish have to be established to exploit the full potential of zebrafish genetics for the study of early vertebrate embryogenesis. We reintroduced a marked zebrafish gene into the germ line. This allowed to investigate whether it is at all possible to efficiently generate transgenic zebrafish lines which express their transgene. We have obtained two lines of zebrafish which not only stably transmit but also correctly express their transgene in subsequent generations. We are now using the green fluorescent protein as an easily detectable reporter to generate chimerical sequences of fish and heterologous origin. These constructs will allow further advances in the generation of zebrafish that express transgenes in a tissue specific fashion.

S15-46

**IGF-I in the Tilapia, *Oreochromus mossambicus***

D. Loffing-Cueni[1], R. Ermatinger[1], D. Meier[1], W. Kloas[2] and M. Reinecke[1]. Institute of Anatomy, University of Zürich[1] and Institute of Zoology, University of Karlsruhe, Germany[2]

In the course of our phylogenetic analysis of IGF-like peptides we studied the presence of IGF-I in the bony fish *Oreochromus mossambicus*, RT-PCR was performed applying total RNA from liver. After synthesis of cDNA, RT-PCR was performed with primers specific for salmon IGF-I. The amplification product of ~240 bp was sequenced and identified as portion of an unknown member of the IGF-I-family showing ~80% homology to trout IGF-I and ~90% identity to *Cottus scorpius* IGF-I In order to compare gene-expression and localization of IGF-I, RT-PCR as well as immunohistochemical methods were used. By RT-PCR a ~240 bp product was detected in liver, pancreas, gut, kidney, gills and brain and verified by southern blotting and hybridization with the sequenced PCR-product of *Oreochromus mossambicus* liver. In accordance, IGF-I-immunoreactivity was localized in cells of pancreas, gut, brain and gills. Thus, IGF-I is expressed not only in liver, but also in various other organs of *Oreochromus mossambicus* indicating additional paracrine actions of IGF-I in bony fish.

S15-47

SEX-PEPTIDE, VITELLOGENIN SYNTHESIS AND UPTAKE

Soller M., Bownes M.*, Kubli E., Zoologisches Institut Universität Zürich-Irchel. *Department of Molecular Biology, University of Edinburgh.

Central to the control of receptivity and oviposition in *Drosophila melanogaster* is the sex-peptide (SP), a 36 amino acid peptide synthesized in the accessory glands. During copulation it is transferred into the female. Injected, or ectopically expressed, SP induces reduced receptivity and increased oviposition. The induced changes imply that the nervous system and (neuro-) endocrine factors are involved in the response of the female to the SP. We have focused on the elucidation of regulatory mechanisms of the SP reaction cascade which leads to the maintenance of the oviposition response. The production of yolk proteins by the fat body and the ovary plays a major part during oogenesis. Quantitative comparison of hemolymph and ovarian yolk protein levels, their rates of synthesis and the levels of yolk protein transcripts in the fat body and ovary revealed new insights into the regulation of vitellogenesis and the underlying hormonal control which might act downstream of the SP.

S15-48

A NEW ENZYME INVOLVED IN MELATONIN PRECURSOR SYNTHESIS IN *DROSOPHILA MELANOGASTER*

R. Amherd, E. Hintermann and Urs A. Meyer, Dept. Pharmacology, Biozentrum, University of Basel, CH-4056 Basel

Transduction of photoperiodic information into hormonal signals involves arylalkylamine *N*-acetyltransferase (aaNAT) activity as the rate limiting step in melatonin formation. In our laboratory an aaNAT from *D. melanogaster* has been purified and characterized [FEBS, 375, 148-150, (1995)]. The cDNA was obtained by screening of a *D. melanogaster* cDNA library with probes derived from internal amino acid sequences. This cDNA was used: 1) For overexpression of aaNAT in *E. coli* and generation of an antibody in rabbits. Western blots with the anti-aaNAT antibody in *D. melanogaster* homogenates showed no correlation of the recognized protein band with the diurnal changes in melatonin levels. 2) Low stringency hybridization of the cDNA library with the original cDNA revealed additional aaNAT clones. One of these clones had sequence differences in the 5' flanking region when compared to the original aaNAT cDNA. On expression in COS cells the clone apparently had higher acetylation activity.
Present studies are directed at characterizing the aaNAT gene(s) and to determine if the additional clones are splice variants or products of separate genes.

S15-49

**A UNIVERSAL PROMOTER FOR DEVELOPMENT AND AGING IN *DROSOPHILA***

F. Donati, R. Ackermann, and Ch. Brack; Biozentrum der Univ. Basel, Klingelbergstr. 70, CH-4056 Basel

For many experiments involving expression of transgenes in *Drosophila melanogaster* a strong promoter, ubiquitously active in all developmental stages and in aging, is required. Such promoters may be derived from promoter sequences of house keeping genes, such as the F1 EF-1α gene. Active protein translation is required in the rapidly dividing cells of the embryo, and the fast growing cells of larvae and pupae. The EF-1α gene is supposed to be very efficiently transcribed in all of the cells of the developing fly. We have investigated the promoter region of the *Drosophila* F1 EF-1α gene during development, and defined a 5 kb promoter fragment, which was able to rescue an EF-1α F1 mutant. This 5 kb fragment was shown to have strong promoter activity in *Drosophila* Schneider cells, in embryos, and larval tissues. There is only little cell division activity in the adult fly, but active protein synthesis is still needed for the survival of the mainly post mitotic organism. The 5 kb promoter fragment was shown to be able to induce transcription in adult flies also. Of particular interest is the finding that the 5 kb fragment shows the same activity in young and old flies, suggesting that this promoter could also be used to express genes in aging flies.

S15-50

**STAGE SPECIFIC & ANTI-SENSE TRANSCRIPTION IN THE HISTONE H1 LOCUS OF *LEISHMANIA MAJOR***

Tanja Noll, Chantal Desponds, Theresa Glaser and Nicolas Fasel, Institut de Biochimie, UNI Lausanne.

The Sw3 gene identified in the protozoan parasite *Leishmania major* encodes for a histone H1 gene. The encoded protein is of unusually small size (105 a.a.) and lacks the central globular domain of histone H1 found in higher eucaryotes but diplays typical H1 characteristics. Nuclear localization was confirmed by immunofluorescence. Both the histone H1 and its polyadenylated mRNAs are accumulating during promastigotes growth in vitro. 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 as well as related proteins were detected *in vivo* suggesting transcription in both directions. Detection of anti-sense transcripts and related proteins in other *Leishmania* species indicates that transcription of both DNA strands may be a wide-spread phenomenon in Trypanosomes.

S15-51

**THE GENOMIC ORGANIZATION OF THE LEISHMANIA HISTONE-H1 LIKE ENCODING GENE SW3**

**A.Formenton, A.Ivens*, N.Saravia°, N.Fasel & S. Belli. Biochem. Inst., UNIL, Epalinges; *Imperial College, London, UK; CIDEIM, Cali, Colombia°**

**The histone H1-like encoding gene SW3 is differentially expressed in *Leishmania*. PCR of genomic DNA using primers to the SW3 ORF gives rise to three products (A1, A2, A3) in *L.major*, which differ in size by ~27 bp. A2 is identical to the SW3 sequence, and A3 is missing a ~27 bp repeat unit. SW3 and A1-A3 are localized to a chromosomal band of ~1.5 Mb band in *L.major*. Within the Braziliensis complex and *L.donovani* the localization of SW3 varies. At least three copies of SW3 exist in the genome of *L.major* as determined by restriction mapping. Analysis of cosmid clones containing the SW3 gene suggests that these copies possess different 3' flanking sequences, and possibly small sequence variations within their ORFs. Future experiments will be designed to understand whether the differential expression of SW3 within promastigotes and amastigotes is influenced by the silencing of one or more of these copies.**

S15-52

**SEPARATION-OF-FUNCTION MUTATIONS IN THE XRN1 (SEP1) EXONUCLEASE OF *S. CEREVISIAE***

J.A. Solinger and W.-D. Heyer. Institute of General Microbiology, Baltzer-Str. 4, 3012 Bern

*S. cervisiae* Xrn1 (a.k.a. Sep1, Kem1, Stpβ/*DST2*, Rar5, Ski1) is a 5'-3' exonuclease active on different RNA and DNA substrates with a yet unexplained specificity for G4 tetraplex structures. Deletion of the *XRN1* gene results in pleiotropic phenotypes including meiotic arrest, multiple defects in microtubule mediated processes, and several defects in rRNA and mRNA turnover events. A genetic strategy has been used to define whether the protein has one or several separable roles in the cell. Genetic and biochemical analysis suggests a role in RNA turnover and points to a connection with the microtubular cytoskeleton and possibly to a role in telomere metabolism. We have now isolated specific point mutations that abolish the exonuclease activity of Xrn1 *in vivo* probably affecting the reaction center. These mutations show separation-of-function. The protein seems to retain a role in meiosis that is not dependent on the exonuclease activity. This may be due to the involvement of Xrn1 in cytoskeletal functions and/or telomere metabolism. Presently, we are purifying and characterizing such mutated emzymes biochemically.

S15-53

**CHARACTERIZATION OF COMPONENTS INVOLVED IN 3' PROCESSING OF MAMMALIAN PRE-mRNAs**

A. Jenny, U. Rüegsegger, S. Barabino, K. Beyer, W. Keller, Biozentrum, CH-4056 Basel.

3' processing of mammalian pre-mRNA proceeds in two tightly coupled steps, cleavage and polyadenylation: cleavage of the primary transcript is catalyzed by CF $I_m$ and CF $II_m$, CstF, CPSF and PAP, polyadenylation requires CPSF, PAP and PAB II. Starting with HeLa nuclear extract, we have separated CF $I_m$ from CF $II_m$ and purified CF $I_m$ to near homogeneity. Purest fractions contain three polypeptides with molecular masses of 68 kDa, 59 kDa and 25 kDa, all of which can be cross-linked to RNA. Investigation of the RNA-binding specificity of purified CstF by the SELEX procedure resulted in sequences which were able to function as downstream sequence elements in *in vitro* cleavage reactions. Furthermore, we have cloned the 73 kDa subunit of bovine CPSF from an expression library. Its open reading frame of 684 aa codes for a protein with a molecular weight of 77.5 kDa. The N-terminal 500 aa show 53 % identity to an unknown, essential yeast protein.