Symposia

S1 1

CLONING AND CHARACTERISATION OF THE cdc 16 GENE OF S. POMBE

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The cdc 16 gene function of the fission yeast S.Pombe is required late in the cell cycle. Temperature sensitive mutants complete s phase and mitosis but fail to complete cytokinesis. The cells initiate multiple rounds of septum formation but do not cleave to produce daughter cells, and are considered to be defective in a control which presents synthesis of more than one septum in each cell cycle. We have cloned the cdc 16 gene by rescue of mutant function. Results of the analysis of the gene and its expression will be presented.

S1 2

THE YEAST *PRP20* GENE IS HOMOLOGOUS TO THE HUMAN GENE *RCC1* AND MIGHT BE INVOLVED IN THE CONTROL OF MITOSIS

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We have isolated a temperature sensitive yeast mutant (*prp20*) which shows a pleiotropic phenotype upon shift to non-permissive temperature. Transcription initation/termination and pre-mRNA processing are affected. In addition, we observe a change in the nuclear structure induced by the temperature shift. Light microscopy studies indicate that mitosis might be affected in the mutant.The wild-type *PRP20* gene was isolated. It encodes a polypeptide of 52kD with a significant homology to the human *RCC1* protein (involved in the control of chromosome condensation). Two potential phosphorylation sites for the *CDC2/CDC28* protein kinase were found in the *PRP20* sequence, leading to the possibility that the *PRP20* protein is regulated by the *CDC2/CDC28* protein kinase.

S1 3

GENES AND PROTEINS INVOLVED IN YEAST DNA REPLICATION

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We have used a combination of genetic and biochemical approaches to define the structure and function of the yeast DNA polymerase-primase complex. This highly conserved protein complex is composed of four polypeptides, called p180,p74, p58 and p48. DNA polymerase is associated with the p180 polypeptide, wherease both p48 and p58 partecipate to the formation of the primase active center. The genes coding for these three polypeptides (POL1,PRI1,PRI2) have been cloned and sequenced. These genes are unique in the haploid yeast genome, essential for cell viability and the level of their transcripts fluctuate during the cell cycle. We are characterizing temperature-sensitive mutants in the DNA polymerase and DNA primase genes to study their physiological function and to define their functional domains. We are also analyzing the molecular mechanisms controlling the expression of the polymerase-primase genes during the mitotic cell cycle.

S1 4

REGULATION OF EUKARYOTIC DNA REPLICATION: SV40 AS A MODEL SYSTEM

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Simian Virus 40 (SV40) is a good model system to investigate elements of the eukaryotic replication apparatus. The virus genome encodes an initiation protein, termed large T antigen which is absolutely required for the induction of viral DNA replication. This protein binds to and unwinds the double-stranded DNA region at the origin of replication and serves, in later steps of the replication cycle, as a DNA helicase at replication forks. The replicative function of the protein is regulated by phosphorylation events. One particular phosphorylation site (Thr124) plays a key role in the activation of T antigen. SV40 mutants with intact DNA binding and intact helicase domains but altered phosphorylation sites are poor initiators of DNA replication. And one reason for this may be the inability of these mutants to form functional initiation complexes. The unwinding of the origin region induces superhelical tensions in the topologically fixed circular DNA molecule. These topological changes are relaxed by DNA topoisomerases which therefore constitute an important part of the initiation pathway.

S1 5

CHROMATIN ASSEMBLY *IN VITRO* DOES NOT DEPEND ON DNA REPLICATION.

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SV40 DNA replicates efficiently in cytosolic extracts from SV40 infected CV1 cells as well as in extracts from human 293 cells supplied with SV40 T-antigen. Addition of nuclear extract leads to chromatin assembly of the DNA. This assembly process is studied by analyzing the psoralen crosslinking pattern and the topoisomerase I induced supercoiling of pre-, post- and replicating SV40 molecules. We see that pre- and postreplicating molecules are packed into chromatin with similar efficiencies, leading to a heterogenous population of reconstituted molecules. In replicating molecules the numbers of reconstituted nucleosomes on the parental and the newly replicated strands are similar. These observations suggest that replication and chromatin assemby of SV40 DNA in cell extracts are not coupled. However, a statistical analysis of the contour lengths of the nucleosomal DNA shows a broad and asymmetric distribution in prereplicative and bulk chromatin, whereas the distribution is narrow in postreplicative strands (like in vivo chromatin). These observations indicate that DNA replication in vitro does not influence the extent of chromatin assembly but it appears to promote the precise DNA folding in the nucleosome.

S1 6

DNA helicase from calf thymus

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DNA helicases are enzymes that transiently abolish the helical structure of the DNA to provide DNA polymerases and RNA polymerases with single stranded DNA. By using a strand displacement assay we have purified a DNA helicase from calf thymus to near homogeneity. In the early steps of the purification this enzyme eluted in the same fractions as DNA polymerase α /primase and was separated from these on a heparin-Sepharose column. Further purification steps included ATP-agarose, FPLC Mono S and glycerol gradient centrifugation. The DNA helicase from calf thymus has a denatured molecular weight of 47 kDa and a Stokes' radius of about 45 Å. The enzymatic activity is dependent on divalent cations especially Mg^{2+} while Mn^{2+} and Ca^{2+} are less efficient. Hydrolysis of ATP and dATP as well as CTP and dCTP support the strand displacement reaction while GTP, UTP, dGTP and dTTP do not. Non-hydrolyzable ATP analogs are not effective, suggesting that hydrolysis of nucleotide 5'triphosphate is required for calf thymus DNA helicase action.

S2 1

GENES REQUIRED FOR RETRIEVAL OF RESIDENT ER PROTEINS FROM THE GOLGI, Hugh R.B. Pelham, Neta Dean, Kevin G. Hardwick, Michael J. Lewis, Jan Semenza and Deborah J. Sweet, MRC Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 2QH, England

Resident proteins of the ER lumen are retained in cells by a mechanism that involves a C terminal signal sequence (KDEL in animal cells, HDEL in *Saccharomyces cerevisiae*). The retained proteins are able to leave the ER and, in yeast, are subject to Golgi-specific carbohydrate modifications. Acquisition of these modifications is dependent on the *SEC18* gene product, which is required for fusion of transport vesicles with their target membrane; thus, sorting of ER residents from secretory proteins occurs in a compartment topologically distinct from the ER.

Several yeast genes that affect the sorting process have been identified. These include some of the *sec* genes that are required for ER-to-Golgi transport (and probably for other transport steps). The absence of one gene (*erd1*) causes a defect in the Golgi that affects the normal modification of glycoproteins and interferes with the retrieval process. Another gene (*erd2*) appears to be required specifically for the transport of HDEL-containing proteins from Golgi to ER; overexpression of this gene causes retrieval to occur from an earlier point on the secretory pathway than normal. *ERD2* encodes a 26 kD hydrophobic, probably integral membrane protein that is essential for growth.

S2 2

ROLE OF MICROTUBULES IN THE ORGANIZATION OF THE GOLGI APPARATUS

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The Golgi apparatus is a compact structure which colocalizes with the microtubule organizing center (MTOC) in a perinuclear region of fibroblasts. This specific location of the Golgi apparatus depends on intact interphase microtubules but not on microfilaments or intermediate filaments. Movement of Golgi elements along individual microtubules has been visualized after removal of the microtubule depolymerizing drug nocodazole and subsequent reclustering of the scattered Golgi elements (Ho et al., Eur. J. Cell Biol. 48: 250-263, 1989). We have identified with a monoclonal antibody a 110kD protein which is associated with the cytoplasmic face of Golgi membranes. This protein interacts with taxol-polymerized microtubules *in vitro* and may thus be involved in linking the Golgi apparatus to the microtubule network and the MTOC *in vivo* (Allan and Kreis, J. Cell Biol. 103: 2229-2239, 1986). We have cloned and sequenced this protein. The functional domains of the 110kD protein are currently being characterized by several *in vitro* and *in vivo* assays.

S2 3

BREFELDIN A INDUCES A MICROTUBULE-DIRECTED BACKFLOW OF GOLGI TO ER MEMBRANES.

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Brefeldin A (BFA) has been shown to induce a secretion block by rearrangement of Golgi elements into the ER. Here, we show that BFA induces a rapid, reversible rearrangement of trans Golgi cisternae along microtubules within ten min followed by a slower fusion into ER membranes. Nocodazole, a microtubule-disrupting agent, inhibited backflow while CCCP, a secretion blocker at the exit of ER, inhibited reassembly of Golgi after BFA removal. Reassembly of Golgi was possible in presence of cycloxeximide.

Pulse/chase studies of galactosyltransferase (GT) in presence of BFA revealed a form of GT intermediate between precursors and mature forms, sensitive to Endoglycosidase H (Endo H) and O-glycanase; Endo H resistance was not observed. These data indicate early O-glycosylation of GT and abolished or reduced complex glycosylation.

These data suggest the existence of a constitutive pathway of Golgi to ER mediated by microtubules and unravelled by BFA by an as yet unknown mechanism.

This work was supported by grant 3.136.088 of the SNSF.

S2 4

IDENTIFICATION OF A 42 KD MITOCHONDRIAL OUTER MEMBRANE PROTEIN AS A COMPONENT OF THE PROTEIN IMPORT SITE.

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Precursor proteins contain discrete targeting sequences that direct them to the correct cellular organelle; the mechanism of the transmembrane movement of a precursor protein is still not understood. In order to identify components of a transport machinery in mitochondria we used a transport intermediate that is stuck in the import site. This intermediate was generated with a chimeric precursor composed of three major parts: (1) a mitochondrial presequence; (2) mouse dihydrofolate reductase (DHFR); and (3) bovine pancreatic trypsin inhibitor (containing three internal disulfide bridges) chemically coupled to the C-terminus of DHFR. The resulting chimeric precursor "jammed" mitochondrial protein import sites since it blocked import of three authentic mitochondrial precursor proteins. We constructed a similar chimeric precursor which carried a photoactivatable group in the crosslinker connecting the trypsin inhibitor to DHFR. Upon illumination, the stuck precursor was crosslinked to a 42 kD outer membrane protein which we named "import site protein 42" (ISP 42) since it is in close contact to the transmembraneous precursor. Antibodies against ISP 42 inhibit protein import into mitochondria.

S2 5

REGULATION OF INTESTINAL LACTASE-PHLORIZIN HYDROLASE

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Lactase-phlorizin hydrolase (LPH) splits lactose, the sugar of milk. In mammals, including many human populations, intestinal LPH activity is high in the suckling and decreases to low levels in adults. We investigated this decline in rabbits, rats, and humans.
In rabbits and rats, LPH mRNA is high at 10 days and declines at weaning. In adults, however, LPH mRNA is again high (whereas enzymatic activity remains low). Similarly, in man no clear difference was found at the RNA level between adults with low *vs.* high LPH activity. There is clearly control at a post-transcriptional level. Possible mechanisms include: (i) Alternate splicing or RNA editing. Comparison of LPH cDNA clones from baby and adult rabbits provided no evidence for alternate splicing. Data for humans are not yet available. (ii) An altered processing of LPH (more rapid turnover, altered glycosylation, altered sorting, etc.). Indeed, there is some evidence for an unusual intracellular high-molecular weight form of LPH in adult rat intestine. In the human case, we asked whether the difference between lactose-tolerant and lactose-intolerant individuals might be associated with differences in the primary sequence of LPH. The complete intron-exon organization, including the sequences of all 17 exons, was established for the cloned chromosomal LPH gene of a tolerant subject. Using the polymerase chain reaction, we could directly sequence the exons of an intolerant subject. Except for silent mutations, the sequences of both subjects were identical. Deciding whether RNA or protein processing is central clearly requires data on human cDNAs.

S3 1

THE STRUCTURAL BASIS OF ANTIGEN-ANTIBODY RECOGNITION

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Most of the methods used for mapping the antigenic sites of proteins are based on functional binding measurements and analyze antigenic cross-reactivity instead of antigenic reactivity. These methods study for instance the cross-reactive binding of peptide fragments with anti-protein antibodies, of proteins with anti-peptide antibodies and of point mutants with anti-wild type antibodies. These methods lead to the view that between 1 and 5 residues are critical to antibody binding. In contrast, X-ray crystallography of antigen-antibody complexes analyzes homologous reactions and shows that about 15 residues of the antigen are in contact with the combining site of the antibody. This apparent contradition regarding the size of the antigenic site can be resolved by taking into account the operational nature of binding measurements.

S3 2

THE ANTIGENICITY OF NATIVE AND DENATURED PROTEIN ANTIGENS

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Often the native form of a protein is unavailable and one has to use the denatured protein or its fragments to raise antibodies. However, the antigenicity of the denatured protein may differ from that of the native form even though both have the same amino acid sequence. The reason is that epitopes are three-dimensional structures. Polyclonal and monoclonal antibodies against native yeast cytochrome c and its heme-free apo-form have been produced and their epitopes mapped by different methods. The heme-free protein is a random coil whereas native cytochrome c has a remarkably stable ordered structure, even when emulsified in complete Freund's adjuvant. Using appropriate mapping techniques one can show that both, antibodies against the native and the denatured antigen are directed against conformation-dependent epitopes which may be assembled from distant parts of the amino acid sequence. The observation of "sequential" epitopes depends more on the mapping technique than on the actual structure of the epitope.

S3 3

OCTAMER-BINDING PROTEINS, A FAMILY OF DNA-BINDING TRANSCRIPTION FACTORS

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Antibodies are produced exclusively in the B lymphocytes. The expression of the antibody encoding genes, the immunoglobulin (Ig) genes, is also restricted to B-cells. The octamer sequence ATGCAAAT is present in the promoter and the enhancer of Ig genes and plays an important role in its tissue-specific expression. This sequence motif is a binding site for nuclear proteins, the so-called octamer transcription factors (Oct or OTF factors). The Oct-1 protein is present in all cell types analyzed so far, whereas Oct-2A and Oct-2B are found mainly in B lymphocytes. All three proteins show the same sequence specificity and binding affinity. It appears that the B-cell specific expression of Ig genes is mediated at least in part by cell type-specific Oct factors and that there are both quantitative and qualitative differences between Oct-1 and Oct-2 factors. Recently, a number of other octamer factor variants were identified. Many of these may be created by alternative splicing of a primary transcript of one Oct factor gene and may serve a specific function in the fine tuning of gene expression.

S3 4

DESIGNED POLYPEPTIDES FOR THE STUDY OF PROTEIN-NUCLEIC ACID RECOGNITION

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This work which is aimed at the construction of polypeptides that may provide "intracellular immunity" by binding tightly to control regions of viral replication, is based on the x-ray structure of the 434 repressor-operator complex. In this model system, the operator sequences ACAAT and ACAAG are recognized by one of the helices at the NH_2-terminal domain of 434 repressor (residues 28-36, QQSIEQLEN). By attaching positive charges to both ends of this recognition helix we obtained tight-binding 42-residue polypeptides possessing almost unaltered binding specificity. ACAAG is also found in the long terminal repeat of HIV where it is followed by GGACTTT. The sequence of this dodecamer is almost identical with that of core enhancer II so that blocking this site by an artificial repressor may reduce the rate of viral replication. We designed an ACTTT-binding helix which was linked via a spacer to the ACAAG-binding helix. Addition of positive charges at both ends resulted in a 60-residue polypeptide that was shown by DNA foot-printing to possess the proposed binding specificity.

S4 1

APPLICATION OF DYNAMIC PROGRAMMING TO PROTEIN STRUCTURE

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The technique of Dynamic Programming optimisation (often better known as the Needleman & Wunsch method) has been applied for many years to the problem of biological sequence comparison. The method was until recently restricted to comparing one-dimensional (sequential) objects but has now been extended by the authors to the comparison of three dimensional objects, specifically, protein structures. This new method works by reducing the three dimensional problem to a series of one dimensional comparisons. The protein structures are firstly reduced to distance plots (two dimensions) then each column of the two distance plots are compared between the two proteins and a consensus alignment is built up. The method has been applied successfully to the comparison of protein structures including some very remotely related pairs. Futher Developments of the method will be described, including the incorporation of additional data (hydrogen bonds, torsion angles, etc.), a new fast algorithm and its extension to multiple structure comparison. New applications of the method, including the comparison of protein structure directly to NMR and crystallographic data, will also be briefly described.

S4 2

MOLECULAR FLEXIBILITY - A PERSISTING CHALLENGE TO MOLECULAR MODELLING

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Facilities for quick generation, manipulation, relaxation as well as examination and comparison of complex three-dimensional molecular structures are essential prerequisites in modern structure-oriented chemical research. They are provided by our in-house computer modeling systems RIMG (Roche Interactive Molecular Graphics) and MOLOC (MOdeling on LOw-Cost terminals). These are highly functional expert systems which are built around powerful generally applicable and robust united-atom force field methods. They are equipped with novel algorithms for quick generation of complete sets of low-energy conformations, structural screening procedures, and conformational set manipulation facilities so that conformation space can be efficiently searched, large sets of structures can be quickly screened by a variety of criteria, and opportunities for structural fixation or conformation induction can be identified. Use of these modeling facilities will be illustrated by various examples related to ongoing research projects.

S4 3

PREDICTING THE TERTIARY STRUCTURE OF ENZYMES FROM SEQUENCE DATA

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Models for the tertiary structure of an enzyme can be built from a set of homologous sequences by analyzing the pattern of divergence within the set. (Benner, S.A., *Adv. Clin. Enzymol.*, **28**, 219-236 (1989)) This approach relies on an understanding of the relative impact of natural selection and neutral drift in divergent evolution. Residues on the surface of the enzyme are identified with ca.94% accuracy by algorithms that detect neutral variation between closely homologous sequences. Residues lying inside are identified (with ca.90% accuracy) by patterns in their conservation. Other algorithms parse the alignment into secondary structural units. From this information, a secondary structure can be assigned with greater than 90% accuracy; other algorithms assemble secondary structural units to yield a tertiary structural model. The use of these procedures to build models for enzymes with unknown structure is discussed.

S4 4

TERTIARY STRUCTURE MODELLING BY INTERACTIVE GRAPHICS COUPLED WITH DISTANCE GEOMETRY OR CONSTRAINED MOLECULAR DYNAMICS

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Information pertaining to protein secondary and tertiary structure can be obtained by inspection of aligned homologous sequences. For example, conserved hydrophobic residues and the occurrence of Pro and Gly are often consistent with only one of either helix, strand or loop structures. Using a graphics computer, preliminary models are constructed in which it is attempted to pack the hydrophobic surfaces of regular secondary structures together, the loops constraining the number of packing orientations. These models are then tested and refined by constrained MD or DG. This approach will be illustrated with a model for the "zinc finger" motif and models for other protein domains which are currently being developed.

S4 5

COMPUTER-SIMULATION STUDIES OF CALMODULIN AND TROPONIN-C

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Recent small angle x-ray scattering studies suggest that bovine brain calmodulin (CAM) and chicken skeletal troponin-C (TNC) may be more compacted in solution than the x-ray structure. The compaction is assumed to be caused by a kinking or bending of the α helix linking the two domains. Molecular dynamics has been used to study the consequences of such compaction on the molecular structure of CAM and TNC and its relevance for inhibitor binding. Different solvent models were tested by including or excluding crystallographic waters in combination with a constant or linear distance dependent dielectric permittivity, ε. Compacted structures were found for both proteins which conserve intradomain structure with the solvent model which includes the waters and takes ε=r. The new structures were shown to be stable by extending the runs to ca. 300ps, and analysis showed that both secondary and tertiary structure including H-bonding patterns were conserved.
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S5 1

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Adhesion during normal development and malignancy

S5 2

WHAT MAKES THE DIFFERENCE BETWEEN TENASCIN AND FIBRONECTIN ?

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Tenascin and fibronectin are both large extracellular matrix proteins with disulfide-linked subunits of about 220kD built from repeated structural and functional domains. About half of each protein consists of socalled fibronectin type III repeats, whereas the structural units of the other halves are different. The functions of most of the type III repeats is unknown, however, in each molecule one particular type III repeat carries the major cell binding site. In fibronectin this site has the sequence GRGDSP, whereas in tenascin the binding site does not contain RGD. For fibronectin the corresponding cellular receptor is the $\alpha_5\beta_1$ integrin, whereas the tenascin receptor remains to be identified. Cells spread out on fibronectin, whereas on tenascin they remain rounded. Furthermore tenascin inhibits cells to spread out on fibronectin. Therefore these two molecules may complement each other in determining cell shape and thus contribute to differentiation and morphogenesis during development.

S5 3

INHIBITION OF TYPE VI COLLAGEN SYNTHESIS IN TUMOUR CELLS

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Type VI collagen is a major constituent of the extracellular matrix of mesenchymal cells. The protein is composed of three different polypeptide chains that form a heterotrimeric molecule with a central triple helix and large globular domains at both ends. The triple helix contains more than ten Arg-Gly-Asp tripeptide units, some of which are likely to be used as cell binding sites. The globular domains are composed of several homologous cassettes which reveal a striking similarity to the collagen-binding motifs found in von Willebrand factor. It is possible that mesenchymal cells bind via their integrin receptors to the triple helix of type VI collagen and that in turn type VI collagen anchors these cells to the interstitial collagen fibres via its collagen-binding domains. Type VI collagen is specifically down-regulated in fibroblasts transformed by DNA or RNA tumour viruses and in cells derived from spontaneous tumours. When introduced into normal cells by viral vectors the activity of a single oncogene product is sufficient to inhibit type VI collagen expression. A reduction in the biosynthesis of this collagen might have dramatic effects on the adhesive properties of the cells and could contribute in this way to the unrestricted growth of tumour cells.

S5 4

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Urokinase-type plasminogen activator mRNA in implanting murine embryos

S5 5

REGULATION OF TUMOR CELL INDUCED EXTRACELLULAR MATRIX DEGRADATION BY URINARY-TYPE PLASMINOGEN ACTIVATOR (u-PA) AND PLASMINOGEN ACTIVATOR INHIBITOR TYPE 1 (PAI-1) GENES.

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The human urokinase-type plasminogen activator (u-PA) gene was transfected into mouse L cells, allowing the isolation of a clone (LuPA) expressing high levels of plasminogen activator (PA), identical to human pro-u-PA in enzymatic, electrophoretic and antigenic properties. The phenotypic changes associated with pro-u-PA expression were analyzed in vitro by degradation of ^{3}H-labeled extracellular matrix (ECM) and invasion of a matrigel basement membrane analogue. LuPA and reference HT-1080 fibrosarcoma cells, in contrast to control Lneo cells degraded the ECM and invaded the matrigel basement membrane and these events could be prevented using anti-u-PA IgG or aprotinin. In addition, we have established a cell line line (LPAI) producing high levels of biologically active plasminogen activator inhibitor type 1 (PAI-1) by transfection of a human PAI-1 cDNA clone in mouse L cells. Coculture experiments demonstrated that LPAI cells efficiently inhibited ECM degradation by LuPA cells or Co-115 human colon carcinoma cells. These results indicate that u-PA expression is sufficient to confer to a cell an experimental invasive phenotype and that PAI-1 may play a critical role in the regulation of such events.

Supported by the Swiss Science Foundation.

S5 6

GLIOBLASTOMA INFILTRATION INTO CNS TISSUE IN VITRO: THE ROLE OF A METALLOPROTEASE.

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Our results indicate that glioblastoma cell invasion into CNS tissue in vitro occurs by means of a novel metalloproteolytic activity, which seems to act on the membrane-bound inhibitory proteins NI-35 and NI-250. These latter inhibitory substrates play a crucial role in the lack of regeneration of lesioned fiber tracts in the adult CNS. Their activity can be neutralized by monoclonal antibodies (Neuron 1: 85-96. 1988). Using CNS tissue explants, CNS frozen sections, or CNS myelin, we have shown that the highly invasive rat C6 glioblastoma cells were not inhibited by the myelin-associated inhibitory components (J.C.B. 107: 2281-2291. 1988). Lack of inhibition was due to a specific mechanism as certain metalloprotease blockers impaired C6 cell spreading on CNS myelin as well as C6 infiltration into CNS explants. A similar mechanism was recently found in a number of human glioblastoma lines, and its occurrance correlated well with the in vivo malignancy of these cell lines.

S6 1

SIGNAL TRANSDUCTION AND ADP-RIBOSYLATION OF G PROTEINS

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Guanine nucleotide-binding (G) proteins that couple cell surface receptors to effectors consist of α, β, and γ subunits in which the α subunit binds GTP. The G protein is active with GTP, and inactive with GDP, bound; Gα (GTP) is inactivated by an intrinsic GTPase. Pertussis (PT) and cholera (CT) toxins catalyze the ADP-ribosylation of specific Gα subunits. ADP-ribosylation by PT blocks G protein pathways by uncoupling G protein from receptor, thus preventing signal transmission. CT-catalyzed ADP-ribosylation activates the G protein pathway (e.g., activation of adenylyl cyclase by Gsα) by inhibiting GTPase, thus preserving Gα(GTP). CT activity is enhanced by 20 kDa guanine nucleotide-binding proteins termed ADP-ribosylation factors (ARFs). ARFs, in the presence of GTP, are allosteric activators of CT, increasing its affinity for substrates; they are highly conserved proteins of ~181 amino acids with consensus sequences for GTP-binding. As both the CT substrate (Gsα) and activator (ARF) require GTP for activity, a GTP-binding protein cascade may be involved in activation of adenylyl cyclase by CT.

S6 2

CLOSTRIDIAL ADP-RIBOSYLATING TOXINS

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Botulinum C2 toxin and C. perfringens iota toxin belong to a novel class of microbial ADP-ribosyltransferases which modify actin. The toxins ADP-ribosylate G-actin not F-actin in Arg-177. ADP-ribosylation inhibits the ability of actin to polymerize and decreases actin associated ATPase activity. Moreover, the ADP-ribosylated actin acts like a capping protein to inhibit the polymerization of non-modified actin.
Botulinum C3 ADP-ribosyltransferase, which is structurally unrelated to botulinum neurotoxins modifies the ras-homologous GTP-binding proteins rho and rac. ADP-ribosylation of rho neither affects GTP-binding nor GTPase activity. Because C3 causes morphological changes of cells in culture (FAO), a process which is paralleled by disappearence of microfilaments, it is suggested that the C3 substrate is involved in regulation of the microfilament network.

S6 3

ADP-RIBOSYLATION AND MITOCHONDRIAL FUNCTION

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Rat liver mitochondria transfer monoADP-ribose (mADPR) from NAD^+ to at least 3 classes of acceptor proteins. A 32 kDa acceptor is located in the inner membrane. Its mADPR modification turns over rapidly. Mitochondria release Ca^{2+} when intramitochondrial NAD^+ is hydrolyzed to mADPR and nicotinamide. There is strong evidence that a physiologically relevant Ca^{2+} release pathway in mitochondria is regulated by monoADP-ribosylation. Recently, several inhibitors of the mADPR-linked Ca^{2+} release were identified. The anti-tumor drug meta-iodobenzylguanidine, a substrate in the cholera toxin-catalyzed ADP-ribosylation, inhibits Ca^{2+} release without affecting NAD^+ hydrolysis. The immunosuppressive drug cyclosporine A and 4-hydroxynonenal, a cytotoxic compound formed during lipid peroxidation, inhibit Ca^{2+} release by preventing NAD^+ hydrolysis. Neither compound inhibits NADH oxidation. The relevance of mADPR-linked Ca^{2+} release in cell functioning will be discussed and related to tumor cell killing as well as to hypoxia/reperfusion injury.

S6 4

POLY ADP-RIBOSYLATION AND PROTEIN SHUTTLING ON DNA

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We have determined the molecular mechanisms involved in the interaction of the poly ADP-ribosylation system with DNA and DNA binding proteins. Poly(ADP-ribose)polymerase scans DNA for the presence of nicks and upon activation, modifies itself with negatively charged ADP-ribose polymers in a strictly processive manner. The numbers and size distributions of polymers vary specificallly with the types of DNA binding proteins present in the reaction, suggesting an adaptive response of the polymerase to its protein environment. We have found that histones have a very high binding affinity to certain classes of ADP-ribose polymers. Enzymatic degradation of polymers reestablishes DNA binding of histones. Thus, the enzymes poly(ADP-ribose)polymerase and poly(ADP-ribose) glycohydrolase act synergistically to shuttle proteins off and on the DNA template, thereby regulating access of other DNA binding proteins to the template.

S6 5

POLY ADP-RIBOSYLATION OF CHROMOSOMAL PROTEINS PARTICIPATES IN THE MODULATION OF GENE EXPRESSION BY CLASTOGENIC CARCINOGENS

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Poly ADP-ribosylation establishes a unique link between "genotoxic" DNA damage leading to structural changes of DNA and transitory modulation of gene expression. Following exposure to the clastogenic carcinogens active oxygen and N-methyl-N-nitro-N-nitrosoguanidine(MNNG) ADPR-transferase served as major poly ADPR-acceptor in mouse epidermal cells. Inactivation of ADPR-transferase may prevent excessive poly ADP-ribosylation. Topoisomerase I served as minor acceptor. Its inactivation might retard DNA replication and allow more time for DNA repair. Poly ADP-ribosylation also occurred at histones and, therefore, may modulate local chromatin conformation. Active oxygen and MNNG induce the immediate early genes c-fos and c-myc. The participation of poly ADP-ribosylation in the induction mechanism is suggested since the inhibition of ADPR-transferase by benzamid suppressed the transcriptional induction of c-fos and the consecutive increase in FOS-protein. Poly ADP-ribosylation of FOS and other transcription factors may alter protein/protein and DNA/protein interactions which are regulating the expression of immediate early genes.

S7 1

DO HYPNOTICS ALTER PHYSIOLOGICAL SLEEP ?

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Among the salient features of physiological sleep are the cyclic alternation of nonREM and REM sleep episodes, and the declining trend of EEG slow-wave activity (SWA; 0.75-4.5 Hz) in the course of the sleep period. Four benzodiazepine (BDZ) hypnotics and a non-BDZ hypnotic were shown to exert massive effects on the sleep EEG: SWA was reduced whereas activity in the 11-14 Hz range was enhanced. However, the drugs did neither modify the periodicity of the sleep cycles nor disrupt the declining trend of SWA. The prolongation of REM sleep latency in some of the sleep recordings could be attributed to abortive REM sleep episodes. The results suggest that hypnotics influence the generating mechanisms of the EEG while leaving the physiological sleep processes largely unaffected.

S7 2

Effects of different benzodiazepines on human sleep: agonist and antagonist ligands of benzodiazepine receptors

R.Blois (Geneva)

S7 3

A MOLECULAR GENETIC APPROACH TO THE STUDY OF SLEEP REGULATION

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The study of mammalian sleep has been hampered by the extreme complexity of the central nervous system and by the broad anatomical distribution of the brain centers involved in the regulation of vigilance states. Early sleep-deprivation (SD) experiments have suggested that specific endogenous substances mediate the control of sleep and waking. However, such "sleep-substances" have not yet been unambiguously identified. To obtain new insights into the regulatory mechanisms of the sleep-wake cycle, we have attempted to isolate and characterize specific molecular markers of these behavioural states. Because of the high transcriptional diversity of the CNS leading to the expression of individual mRNA species in discrete brain regions at very low concentrations, we have developed a molecular genetic approach based on subtractive cDNA-cloning. Using these techniques, we were able to detect and isolate in rat forebrain four cDNA-clones whose corresponding transcripts were expressed at a lower level after 24 hr SD, and six cDNA-clones whose corresponding transcripts were expressed at a higher level. The expression of two of the former transcripts was shown to be reduced by 50% after 24 hr SD as well as after 12 hr cold exposure. Under baseline conditions, the level of these transcripts showed a pronounced 24 hr rhythm with the maximum at dark onset, the beginning of the rat's active period. Their primary structure analysis did not reveal any correspondence with transcripts that have been characterized todate. The results of this study, which represents the first attempt to investigate sleep homeostasis at the molecular genetic level, are consistent with an involvement of the cloned transcripts in the regulation of the sleep-waking cycle.

S7 4

MOLECULAR CLONING OF DIURNALLY REGULATED MRNAS FROM THE MURINE SUPRACHIASMATIC NUCLEUS

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The mammalian circadian system is known to have a strong organizing influence on the timing of sleep and wakefulness. The suprachiasmatic nucleus of the hypothalamus has been shown to be a master oscillator capable of generating circadian rhythms both in vivo and in vitro. We therefore sought to identify diurnally varying molecules that function in timekeeping or transduction of circadian information to arousal state. A cDNA library of 1 x 10^6 recombinants in lambda ZAP II was constructed from 40 ug of total RNA form mouse anterior hypothalamus. This library was differentially screened with cDNA synthesized from tissue-punched suprachiasmatic nuclei taken at circadian times 2 and 14. From 200,000 clones screened, 55 plaques were differentially expressed on first round screening; 26 of these showed strong (10-fold) differential hybridization on second round screening. Southern analysis of these clones showed seven independent recombinants displaying diurnal regulation. These clones are presently being analyzed in situ in free-running and entrained animals.

S7 5

Circadian Influences on Sleep Structure and Sleep EEG: Interindividual Differences.

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To examine the influence of the circadian system on sleep organisation, sleep was recorded in 8 Morning-type subjects (M-types) and in 8 Evening-type subjects (E-types) during 3 subsequent nights, and after shifting bedtime to the day, during 3 consecutive days. During night-sleep the most obvious differences between the groups occurred in the first part of the sleep-period: The M-types produced more slow wave sleep (SWS) and less REMS in cycle 1 than the E-types. In the M-types the mean slow wave activity (SWA) decreased monotonically over successive NREMS episodes. However, in the E-types SWA remained at the same level in cycle 1 and 2. Day-sleep 1 resembled night-sleep to a large extent in both the M-types and E-types, which is remarkable, since the preceding waking time was extended. But the sleep organisation of day-sleep 2 and 3 was clearly differently affected by the shift in sleep-time: In the M-types REMS increased and SWS decreased in cycle 1 compared to their night-sleep. In the E-types no such effect was observed. Instead, cycle 1 to 3 were systematically shortened. During day-sleep the decline of SWA was delayed in both groups, caused by relative high SWA in cycle 2 in the M-types and in cycle 3 in the E-types. It was concluded that circadian processes influence the NREMS-EEG. Possibly this effect is mediated by body temperature or by REMS.

S8 1

MOLECULAR ANALYSIS OF *ENHANCER OF SPLIT*, A GENE INVOLVED IN NEUROGENESIS IN *DROSOPHILA MELANOGASTER*

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Neurogenesis in *Drosophila* requires ectodermal cells to choose a neural versus an epidermal developmental pathway. This choice, mediated by cellular interactions, seems to be under the genetic control of the "neurogenic loci". Two neurogenic genes, *Notch* and *Delta*, encode rather large trans- membrane proteins, supporting the hypothesis of their involvement in cell communication. We are interested in *Enhancer of split [E(spl)]* which interacts genetically with *Notch* and *Delta*. *E(spl)* transcripts m9/m10 share homology with mammalian ß- transducin suggesting a role in the transduction of developmental signals generated by the action of *Notch* and *Delta*, respectively. Surprisingly, m9/m10 products are detected in the nuclei of the developing nervous system. Other transcripts in the *E(spl)* region encode putative c-myc like proteins, and therefore might be transcriptional regulators. However, their function remains obscure since no support for a direct transcriptional control of either *Notch* or *Delta* by *E(spl)* could be found. We are currently investigating the role of this class of transcripts in neurogenesis.

S8 2

POSITION DEPENDENT DETERMINATION OF CELL FATE IN THE DEVELOPING EYE OF DROSOPHILA

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The developing eye of Drosophila is one of the few systems in which cell fate decisions can be studied at the single cell level. The determination of the UV-sensitive R7 photoreceptor cell is under the control of the *sevenless* gene. The *sevenless* gene product is a member of the receptor tyrosine kinase family of proteins. It acts as a putative receptor for the R7-inducing signal. When then protein is missing or when a single amino acid substitution inactivates its tyrosine kinase domain the presumptive R7 cell develops into a non-neuronal cone cell. Although expression of *sevenless* is restricted to a subpopulation of ommatidial presursor cells, we have shown that ubiquitous expression of *sevenless* does not alter the spatially restricted formation of R7 cells. We propose that the position-dependent specification of cell fate is controlled by the spatially and temporally restricted expression of the inducing signal and that the presence of a receptor renders the undeterimned cells competent to respond to the inducing signals. We have characterized the cis-acting regulatory elements responsible for the spatially restricted expression of the *sevenless* gene. Using these enhancer sequences we have begun to alter the expression of other genes involved in the specification of cell fate in the eye. Expression of the homeobox gene *rough* in R7 transforms this cell into R1-6 type photoreceptor cell. This indicates that *rough* controls photoreceptor cell identity and that the R7 precursor is multipotent.

S8 3

DO NEURONS AND GLIA DERIVE FROM THE SAME SET OF NEURO-EPITHELIAL CELLS ?

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An old but still open problem in developmental neurobiology of the mammalian central nervous system (CNS) is related to two basic questions: do nerve and glial cells arise from a common cell lineage or do separate neuronal and glial precursors exist early in development and when do they appear ? An original explant culture system was elaborated, using minisegments of newborn rat optic nerves (ON), which are composed of astrocytes, progenitor cells and unmyelinated axons; neuronal cell bodies are absent. In addition to the context of developmental and functional features of glia in vitro in the absence of ganglion cells, these explants gave also rise to a neuron-like cell type, which never occurs in situ. These cells show morphological, fine structural and immunocytochemical characteristics ascribed to differentiated neurons. This observation suggests the existence of a common neuron-glia progenitor in the post-natal ON, or the presence of a small population of neuronal precursors, which never produce glia descendants in situ but is still present late in CNS development. To map the neuron-like cell lineage "downstream", we will combine the technique of retroviral gene transfer with immunocytochemical procedures. NSF 3100-009 237, Swiss MS Society.

S8 4

THE AXONALLY SECRETED PROTEIN AXONIN-1 IS A POTENT SUBSTRATUM FOR NEURITE GROWTH

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Cell adhesion molecules with restricted expression in the membrane of fasciculated axons (AxCAMs) are thought to have a prominent role in the guidance of growth cones in the formation of nerve fiber tracts. A recently characterized AxCAM, membrane-bound axonin-1 (Ruegg et al., J. Cell Biol.,in press) is particularly intriguing in that a structurally highly similar soluble homologue is secreted from axons (Stoeckli et al., Eur.J.Biochem. 180, 249-258, 1989). The present study demonstrates that axonally secreted axonin-1 strongly promotes neurite outgrowth when presented to neurons in an immobilized form. In view of their structural similarity we conclude that secreted and membrane-bound axonin-1 interact with the same growth promoting neuritic receptor, thereby implicating axonally secreted axonin-1 as a regulatory element of growth cone-neurite interactions during neurite growth and/or pathway selection.

S8 5

THE ADHESION MOLECULE ON GLIA (AMOG) IS A HOMOLOG OF THE β SUBUNIT OF THE Na,K-ATPASE

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AMOG is a Ca^{2+}-independent adhesion molecule mediating neuron-astrocyte interaction in vitro. The sequence of mouse AMOG reveals 40% amino acid identity with the β subunit of rat brain. AMOG and the β subunit of brain Na,K-ATPase have identical apparent molecular weights, and are immunologically cross-reactive. Immunoaffinity-purified AMOG is associated with a protein of 100000 apparent M_r. Monoclonal antibodies reveal that this associated protein comprises the α2 (and possibly α3) isoforms of the Na,K-ATPase catalytic subunit. The monoclonal AMOG antibody that blocks adhesion interacts with Na,K-ATPase in cultured astrocytes by its ability to increase ouabain-inhibitable $^{86}Rb^+$ uptake. AMOG-mediated adhesion occurs, however, both at 4°C and in the presence of ouabain. We hypothesize that AMOG or variants of the β subunit of the Na,K-ATPase, tightly associated with an α subunit, are recognition elements for adhesion which link cell adhesion with ion transport.

S8 6

MYELIN-DEFICIENT MICE: FROM PHENOTYPE TO GENOTYPE

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Myelin-deficient (mld) mice are affected by ataxia, convulsions and shortened lifespan. The synthesis of myelin basic protein (MBP) is severely reduced in mld mice due to low MBP mRNA concentrations. Translational and post-translational events are unaffected. The mld Mbp gene is duplicated. Two genes are in tandem position, and the upstream gene contains a large inversion. Both genes are transcribed from their own promoters. The upstream gene gives rise to antisense RNA and is transcribed at a higher rate than the "normal" downstream gene. Both sense and antisense RNA accumulate in mld brain nuclei, but only low amounts of sense RNA are detected in the cytoplasm. Formation of duplex sense:antisense RNA in nuclei could inhibit processing and transport of sense RNA. Since MBP is an important structural protein of myelin, low MBP concentrations impede the compaction and stabilization of large amounts of membranes formed during the active myelination phase. As a consequence, axons are wrapped by few, uncompacted myelin lamellae. But, protracted Mbp gene expression in mld mice provides late and partial correction of the abnormal phenotype.

S9 1

CONFOCAL LASER FLUORESCENCE MICROSCOPY OF BIOLOGICAL SPECIMENS

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Confocal fluorescence microscopes have an improved discrimination against out of focus contributions when compared to conventional fluorescence microscopes. This is due to the fact that a point source and a point detector are used to scan the sample. Confocal images are therefore always recorded in focus. Cell biologists use this capability to record three-dimensional images of fluorescently labeled samples by observing different planes consecutively and generating a three-dimensional view in the computer. The effect of out of focus discrimination is most prominent in thick specimens. The confocal microscopes at the EMBL are therefore mostly used to observe epithelial cells (up to 20 micron high) or, in a different application, fibroblasts undergoing mitosis (up to 8 micron high). In both cases the density of the fluorophore is high and conventional images suffer from a strong flare. This talk will a) give a short introduction to confocal microscopy and b) present some results obtained with confocal microscopy in the cell biological research at the EMBL.

S9 2

DETECTION OF VIRAL PARTICLES IN LIVING CELLS BY VIDEO MICROSCOPY

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The observation of living cells and moving objects by light microscopy can be greatly improved by image processing of video signals in real time. The enhancement of images results in a better recognition of details, the imaging of which is not easily predictable by the criteria of the optical resolution of microscopes. We have been able to analyse the budding of an enveloped virus (respiratory syncytial virus) on the plasma membrane of infected cells by directly visualizing the formation of virus filaments (diameter: 110nm) as well as the process of fusion between viral envelopes and target membranes. Both processes take place with an average speed of 200 nm/sec. Furthermore, we succeeded to detect individual colloidal gold particles (30nm) coated with antiviral antibodies during the progress of the immunoreaction. This method enabled us for the first time to study the kinetics of the binding of antibodies to a surface antigen and represents a new and powerful tool for immunohistochemical investigations under physiological conditions. Videorecordings of viral budding and fusion as well as of the reaction of viral elements with immunogold particles document the usefulness of video microscopy at a quasi-molecular resolution.

S9 3

STEREOSCOPIC VISUALIZATION OF NATIVE DNA.

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The study of the spatial arrangement of DNA by electron microscopy has been impaired by the necessity of drying the specimen and adsorbing it onto a supporting film. We are using cryo-electron microscopy of thin vitrified layer for observing short DNA molecules in their native, fully hydrated state.
Unstained double-stranded DNA molecules can be seen in water if stringent image formation conditions are respected and if the solution is very clean. Stereo-pairs can be obtained in favorable cases and the 3-D path of the molecule can be visualized. Computer image processing is also used for 3-D reconstruction. In general the molecules remain distant from the surfaces of the thin vitrified film. Their super-helicity does not change during specimen preparation but their shape is critically dependant on salt type and concentration in the medium.
The method should be useful for the study of DNA topology, DNA-proteins interactions and gene regulation.

S9 4

Scanning Tunneling Microscopy of Biomacromolecules

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The resolving power of Scanning Tunneling Microscopy (STM) for analyzing structural and electronic properties of metal- and semiconductor surfaces is now well established. On this basis biological macromolecular structures, carefully dehydrated by freeze-drying and coated with a three-dimensionally stable, thin conducting film (Pt-Ir-C) can routinely be analyzed. Recent work on uncoated biological specimens adsorbed to a conducting support, extend the application of the STM to a direct investigation of substance classes classically thought to be non-conductors.
Molecular structure research by means of STM is illustrated on the examples of coated and uncoated recA-DNA complexes (helical intermediate structures in bacterial genetic recombination), DNA and HPI-layer (a regular bacterial surface layer). The STM images are compared to transmission electron microscopical data. In addition reproducibility and accuracy of STM-imaging have been investigated on phage T4 polyheads type III (aberrant bacteriophage capsids), freeze-dried and coated with a thin conducting film. The surface structure has been resolved in spite of it's small corrugation of only about 1 nm, taking advantage of the high resolution power of the STM especially in the z-direction (perpendicular to the support).

S9 5

Biological Perspectives of High Resolution Scanning Electron Microscopy

Martin Müller, Institut für Zellbiologie, ETH- Zürich

Modern Scanning Electron Microscopy (SEM) is now capable of elucidating the surface of biological intracellular structures with a resolution that paralells that of TEM. This level has been achieved through complimentary progress in instrumentation (field-emission guns, "in-lens" type SEMs), specimen preparation (cryotechniques) and improved knowledge of signal generation and detection.

Significant contributions by high resolution SEM are expected through immuncytochemistry applications; precise localization of surface antigens and receptors. Colloidal gold particles, 1nm. in diameter, coupled to antibodies, FAB fragments, or other ligands, can now be unambiguously located, on the surface of biological structures, by highly sensitive backscattered-elelectron detectors. (The labelling precision is limited mainly by the size of the ligand.)

The direct examination of freeze-fractured biological specimens by high resolution SEM is of great practical importance; very large fracture faces can be examined without necessitating the removal of the biological material from the heavy metal replica.

S10 1

BIOCHEMICAL AND GENETIC STUDIES OF THE INTERACTION BETWEEN THE CHLOROPLAST AND THE CYTOSOL

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The major aim of this paper will be to discuss how mutants with a selective and progressive reduction in the activity of a particular enzyme can be used to provide qualitative and quantitative insights into metabolic control.
Experiments will be presented using mutants of Clarkia xantiana with a progressive reduction in the activity of phosphoglucose isomerase in either the plastid or the cytosol. These mutants have allowed us to (a) provide decisive evidence for the role and effectiveness of Fru2,6bisP in mediating a feedback control of sucrose synthesis, (b) investigate the influcence of a decreased rate of sucrose synthesis on starch synthesis and vice versa and (c) estimate the flux control coefficients and and elasticity coefficients for various enzymes in the pathway of sucrose synthesis.

S10 2

INTRA- AND INTERCELLULAR COMPARTMENTATION OF ASSIMILATORY SULFATE REDUCTION

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IT IS GENERALLY ACCEPTED THAT IN LEAVES ASSIMILATORY SULFATE REDUCTION IS LOCALIZED IN THE CHLOROPLASTS, SINCE ALL THE ENZYMES INVOLVED IN THIS PATHWAY HAVE BEEN DETECTED IN THESE ORGANELLES. USING SUCROSE DENSITY GRADIENTS, THE ENZYMES OF THIS PATHWAY HAVE BEEN LOCALIZED IN ROOTS OF YOUNG PEA SEEDLINGS. ATP-SULFURYLASE (EC 2.7.7.4), ADENOSINE 5 -PHOSPHOSULFATE SULFOTRANSFERASE AND SULFITE REDUCTASE (EC 1.8.7.1) CAN BE FOUND ALMOST EXCLUSIVELY IN THE PROPLASTIDS WHILE O-ACETYL-L-SERINE SULFHYDRYLASE (EC 4.2.99.8) IS PREDOMINANTELY PRESENT IN THE CYTOPLASM.
THE INTERCELLULAR DISTRIBUTION OF ASSIMILATORY SULFATE REDUCTION WAS ANALYZED IN THE C_4 PLANT ZEA MAYS L. ATP-SULFURYLASE AND ADENOSINE 5 -PHOSPHOSULFATE SULFOTRANSFERASE ARE RESTRICTED TO THE BUNDLE SHEATH CELLS, WHILE SULFITE REDUCTASE AND O-ACETYL-L-SERINE SULFHYDRYLASE COULD BE DETECTED IN MESOPHYLL CELLS, TOO.

S10 3

ROLE OF THE VACUOLE IN THE STORAGE AND MOBILIZATION OF SOLUBLE CARBOHYDRATES

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The large central vacuole is one of the most distinctive features of mature plant cells. It often occupies as much as 80 to over 90% of the cell volume and fulfills various important functions, such as stabilization of cell shape, osmotic adjustment, storage and mobilization of metabolites and nutrients, detoxification and metabolization. The recent development of refined methods for the isolation and purification of intact vacuoles has allowed a more comprehensive appreciation of the great diversity of their roles. In particular, it has become increasingly evident that the vacuole is a very dynamic organelle. This dynamic quality will be illustrated by discussing the role of the vacuole in storage and mobilization of fructans (polyfructosyl-sucrose) in tubers of Jerusalem artichoke and primary leaves of barley and of stachyose (digalactosyl-sucrose) in tubers of *Stachys sieboldii*. As these carbohydrates are highly water-soluble and may account for up to 70 to 80% of the dry weight it is not surprising that they are stored in the vacuole. In the case of fructans, however, it is particularly interesting that the vacuole is also the site of their degradation and even their synthesis. The most striking feature of *Stachys* vacuoles is their ability to store stachyose in the presence of its degrading enzyme α-galactosidase. Regulatory mechanisms allowing these extravaganzas will be discussed.

S10 4

A vacuole-deficient mutant (end1) of Saccharomyces cerevisiae fails to store arginine and polyphosphate

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Yeast cells grown on arginine as the sole nitrogen source accumulate large quantities of poly-P (1.8 mMol P_i equivalents of poly-P/g protein) and arginine (2.1 mMol arginine/g protein) in their vacuoles. These vacuole storage pools can be used as N- or P-source under nitrogen and phosphate starvation conditions.
We examined the vacuole deficient mutant end1 and found that it stored virtually no arginine and poly-P (<0.1 mMol P_i eq/g protein) when grown on a medium with arginine as sole nitrogen source. When transferred to a nitrogen or phospate lacking medium it stopped growing much faster than the wildtype. The small arginine and poly-P pool present in the mutant changed but little. Addition of P after P-starvation induced no accumulation of poly-P or of additional arginine in contrast to the situation in wildtype cells. Instaed of arginine, which is used as main nitrogen source under N-starvation conditions in wildtype cells, glutamate and glutamine, present in end1 cells at similar levels as in wildtype cells, are rapidly mobilized.

S10 5

The malate carrier of the tonoplast

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Malic acid accumulates in CAM-plants during the night, and this process is reversed during the daytime. In contrast, in leaves of C_3 plants, levels of malate are high at the end of the day and low in the morning. Both examples reflect the dynamics of vacuolar compartmentation.
Recent studies with isolated vacuoles have shown that carriers with very similar properties are responsible for the transport of malic acid into the vacuole of CAM and C_3 plants. Both carriers show an apparent K_m of 1 - 2.5 mM for L-malate uptake and are not specific for the enantiomer of the acid. D-malic acid and other di- and tricarboxylic acids act as competitive inhibitors. Uptake of malate is ATP-dependent, suggesting that the tonoplast-bound ATP-ase is involved in the energization of the transport process. Solubilisation, partial purification and reconstitution of the malate carrier into liposomes allowed us to further characterize this transport system.

S10 6

H^+/Ca^{2+}-ANTIPORT DRIVEN BY THE PYROPHOSPHATE-DEPENDENT TONOPLAST PROTON PUMP

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Calcium uptake by tonoplast enriched membranes vesicles from maize (*Zea mays* L. cv LG 11) primary roots was studied. The pyrophosphate (PPi)-dependent proton pump was used to energize specifically the tonoplast vesicles. The Ca^{2+} uptake was measured using $^{45}Ca^{2+}$, and found to be linear for 10 min when oxalate (10 mM) was present. The Ca^{2+} transport was completely inhibited with proton ionophores (FCCP and monensin), arguing for a Ca^{2+}/H^+ antiport. No interferences due to the ATP-dependent Ca^{2+} pumps from the plasmalemma and the ER were obtained. Membranes were further fractionated using linear sucrose density gradients (10-45%) and identified with marker enzymes. The Ca^{2+} uptake distribution was similar to the tonoplast PPi-dependent proton pumping, pyrophosphatase and ATPase activities: the Ca^{2+}/H^+ antiport is consequently located on the tonoplast.

S11 1

IMMUNOCHEMISTRY AND ULTRASTRUCTURE OF THE ENDOCRINE HEART.

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The prehormones of cardiac peptide hormones are synthesized in myoendocrine cells of the atrial appendages of the heart. Ultrastructural studies reveal a typical peptide-producing intracellular apparatus. Secretion stimulation and inhibition are shown during experimental states of volume load and decrease. Ultrastructural analysis comoined with immunocytochemistry shows the prohormononal character of CDD/ANP (cardiodilatin/ atrial natriuretic polypeptide) within the cells which is also confirmed by extraction studies. During exocytosis, the posttranslational processing of CDD/ANP occurs and causes a release of CDD/ANP-99-126, the circulating form of CDD/ANP. Biochemical and morphological means are advanced to prove the mode of processing of CDD/ANP.
Supported by a fellowship of the VW-Stiftung.

S11 2

EFFECTS OF ATRIAL NATRIURETIC FACTOR ON CULTURED RAT AORTIC SMOOTH MUSCLE CELLS.

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Atrial natriuretic factor (ANF) has been shown to have vasodilatory properties. Receptors for ANF have been characterized in vascular smooth muscle cells (VSMC). In VSMC two main biological responses, namely contraction and prostacyclin production are elicited by angiotensin II (ANG II) stimulation. ANG II raises intracellular free calcium and stimulates protein kinase C (PKC) in VSMC. Phorbol-12-myristate-13-acetate (PMA) directly activates PKC in these cells. We showed that ANF inhibited ANG II- and PMA-stimulated PKC activity by 45% in the cell membrane but did not influence cytosolic PKC activity. ANF significantly inhibited PMA-induced prostacyclin production but did not interfere with ANG II-stimulated prostacyclin production. Thus it appears that when only the PKC-stimulated pathway is activated, the ANF-mediated reduction of membranous PKC activity leads to an inhibition of the final biological response. Our results suggest that ANF is an endogenous inhibitor of the cellular PKC system.

S11 3

EXPERIMENTAL ASPECTS OF ACTION OF ANP ON THE KIDNEY

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A number of in vivo and in vitro experiments have revealed four major effects of atrial natriuretic peptide (ANP) in the kidney, namely i)induction of natriuresis and diuresis, ii)increase of renal blood flow (RBF), iii)increase of glomerular filtration (GFR) and iv)suppression of renin secretion. Possible mechanisms for the natriuretic effect of ANP comprise inhibition of sodium reabsorption in the collecting duct and an increase of GFR. Increase of renal blood flow reflecting a fall of renovascular resistance is probably due to a direct effect of ANP on renal vascular smooth muscle cells. Enhancement of glomerular filtration appears as the result of the increase in RBF and an increase of the glomerular hydraulic permeability. The mechanism by which ANP suppresses renin release could involve a direct effect on juxtaglomerular cells and/or an indirect effect mediated by the macula densa.

S11 4

ATRIAL NATRIURETIC PEPTIDE IN MAN

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The heart is the major source of atrial natriuretic peptides (ANP). A propeptide is stored in atrial myocytes. In normal humans, atrial distension secondary to volume overload and/or increased atrial pressures are thought to stimulate the secretion of biologically active α-ANP (ANF-[99-126], 28 amino residues) into the circulation. Plasma immunoreactive ANP (irANP) rises in response to acute sodium-volume loading, the central shift of volume produced by lying down or by immersion, acute increases in blood pressure (BP), dynamic exercise, or the administration of glucocorticoids or mineralocorticoids. Plasma irANP also rises with aging. Synthetic α-ANP infused acutely i.v. can lower BP, reduce plasma volume by an extravascular shift, cause baroreflex-mediated sympathetic activation, directly inhibit adrenal steroidogenesis and lower plasma aldosterone and cortisol, directly inhibit renal renin release, elevate plasma insulin; diuresis, free water clearance and natriuresis increase already in response to low α-ANP doses that raise plasma irANP within the physiological-pathological range. It follows that in addition to direct influences on cardiovascular and renal function, the ANP system may comprises a cardio-adrenal feedback mechanism and perhaps also modulate insulin and the release of ADH. The major although yet unproven physiological role of the ANP system may be the protection of the heart against volume and/or pressure overload. The pathophysiological, diagnostic and therapeutic aspects of elevated plasma irANP values, ANP measurements, or administration of synthetic ANP, respectively, in various diseases are currently under intense study and of great potential interest.

S12 1

VESICLE TRAFFIC AND PLASMA MEMBRANE BIOGENESIS IN POLARIZED HEPATOCYTES

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We are studying the mechanisms involved in the biogenesis, sorting and delivery of membrane glycoproteins that reside in different domains of the hepatocyte surface. Using in vivo metabolic (pulse-chase) labeling in conjunction with subcellular fractionation and specific protein immunoprecipitation, we have found that 5 newly-synthesized plasma membrane proteins (3 apical and 2 basolateral) are all delivered first to the basolateral domain and then the 3 apical proteins are selectively retrieved and transported to their site of function, the apical domain (J. Cell Biol. 105:1241-51 ('87)). To learn more about the pathway, we have used perturbants of vesicle traffic in hepatocytes to block plasma membrane biogenesis. The goal has been to accumulate new molecules somewhere in the late Golgi or beyond and identify the vesicle compartments and carriers involved. We have found that bile duct ligation blocks delivery of new apical proteins to the apical domain and leads to their intracellular accumulation around dilated bile canaliculi. Colchicine appears to block exit of new apical proteins from the basolateral surface.

S12 2

SINUSOIDAL AND CANALICULAR MEMBRANE TRANSPORT SYSTEMS AND THEIR DRIVING FORCES

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Sinusoidal uptake of bile constituents and of their precursors and their canalicular secretion serve for the biliary elimination of endogenous and exogenous compounds and provide the osmotic driving force promoting canalicular bile flow. Transepithelial osmotic water flow appears to occur through cell membrane water pores whereas electrolytes permeate predominantly across the tight junctions. Organic solutes are transported against considerable transmembrane concentration gradients and specific carrier systems have been identified and characterized, particularly in isolated sinusoidal and canalicular cell membrane vesicles. These include transporters for bile acids, bilirubin, amino acids, organic acids, glutathion, fatty acids or xenobiotics. Energy for concentrative transport is derived from the hydrolysis of ATP either by the carrier itself (primary active) or by Na,K-ATPase. This sinusoidal membrane enzyme operates as the load dependent active component of a pump/leak-system. Carrier mediated coupling of solute transport to the reentry of Na^+ into the cell down its chemical concentration gradient (approx. 10:1) provides for the cellular uptake of most solutes (secondary active) and contributes to establish the transmembrane pH gradient (e.g.Na/H-exchange), a driving force for tertiary active solute transport. K^+-leak out of the cell through membrane K-channels establishes the intracellular negative electric membrane potential which drives electrogenic transport processes, particularly the canalicular extrusion of organic anions. As regulation of cell volume and cell pH depend in part on common transport mechanisms bile formation is modified by these cell functions.

S12 3

THE ROLE OF TWO GENES ENCODING LIVER-ENRICHED TRANSCRIPTION FACTORS IN TERMINAL LIVER DIFFERENTIATION

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The two albumin promoter elements B and D are particularly important for the potent liver-specific albumin transcription. While the element B binds the highly glycosylated liver-specific transcription factor HNF1, element D is a recognition sequence for at least three distinct factors, DBP-1, DBP-2 and C/EBP. While these three proteins appear with distinct developmental profiles, all three of them bind to their cognate DNA sequence via a conserved basic domain and by doing so activate albumin transcription in co-transfection and/or in vitro transcription experiments. In contrast to the homeobox-containing protein HNF1 that is present in fetal, adult and transformed hepatic cells, the D-binding trans-activators accumulate only in terminally differentiated non-dividing hepatocytes and are absent from fetal, regenerating or immortalized hepatocytes. It is therefore tempting to speculate that one or all of these D-binding factors are involved in both, transcription and replication control during liver differentiation.

S12 4

HORMONAL REGULATION OF HEPATIC mRNA LEVELS OF IGF I AND THE FETAL IGF BINDING PROTEIN (IGFBP-2)

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The liver is the primary source of insulin like growth factor I (IGF I) and of the fetal IGF binding protein (IGFBP-2). To examine the hormonal regulation of hepatic mRNA levels for IGF I and IGFBP-2, primary hepatocytes were treated with 10 nM insulin and/or 10 nM growth hormone (GH) for 16 hours and RNA was examined by Northern blotting. Results show, that insulin is a potent inhibitor of IGFBP-2 mRNA, whereas GH has no effect. In contrast, both, insulin and GH, upregulate IGF I mRNA levels. GH is twice as potent as insulin and the respective stimulation factors of insulin and GH are additive if both hormones are used in combination. The ED_{50} for IGFBP-2 mRNA suppression and IGF I mRNA stimulation by insulin was in the range of 1-4 10^{-10} M. These results reveal a novel regulatory link between the two structuraly related hormones IGF I and insulin.

S12 5

EXPRESSION CLONING OF THE HEPATOCELLULAR Na^+-DEPENDENT BILE ACID UPTAKE SYSTEM

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The expression of the basolateral Na^+-taurocholate (TC) cotransport system of rat hepatocytes has been studied in Xenopus laevis oocytes. Injection of rat liver poly(A)$^+$ RNA into the oocytes resulted in the functional expression of Na^+ gradient stimulated TC uptake within 3 to 5 days. This Na^+ dependent portion of TC uptake exhibited saturation kinetics (apparent Km 91 μM) and could be inhibited by DIDS. Furthermore, the expressed TC transport activity demonstrated similar substrate inhibition and stimulation by low concentrations of BSA as the basolateral Na^+-TC cotransport system previously characterized in intact liver, isolated hepatocytes and isolated plasma membrane vesicles. Finally, a 1.5 to 3.0 kb size-class of mRNA could be identified that was sufficient to express the basolateral Na^+-TC uptake system in oocytes. These results demonstrate that "expression cloning" represents a promising approach to ultimately clone the gene and to further characterize the properties of this hepatic membrane transport system.

S13 1

INTRACELLULAR CALCIUM TRANSIENTS OBSERVED IN CEREBRAL ASTROCYTES IN RESPONSE TO EXCITATORY NEUROTRANSMITTERS

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Glial cells are often thought of as passive partners in neuronal function, being responsible mainly for the recycling of ions and neurotransmitters. We find, though, that cerebral astrocytes in primary culture respond to the excitatory neurotransmitter glutamate with a strong increase in the intracellular free calcium concentration ($[Ca^{2+}]_i$). Spike-like increases are observed in response to glutamate and its analogues quisqualate and ibotenate. Removal of external Ca^{2+} has no immediate effect on spike generation in response to application of these agonists, suggesting that the spikes, like those in other cells, result from a release of calcium from intracellular stores by IP_3. Kainate, on the other hand, causes a slower and more maintained increase in $[Ca^{2+}]_i$ which can be rapidly abolished by the removal of external calcium, showing that it depends on a calcium influx across the plasma membrane. Kainate can also activate Ca_o-independent spikes, suggesting that it can also act as an agonist at an IP_3-linked receptor. These experiments demonstrate that excitatory neurotransmitters generate calcium signals in glial cells, and the results have implications for the mechanism of generation of calcium spikes in other cells.

S13 2

RECEPTOR MODULATION OF Ca^{2+} AND K^+ CHANNELS IN EXCITABLE PITUITARY CELLS.

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Modulation of ion channel activity in the plasma membrane is an important element of signalling. Pituitary hormone secretion is essentially controlled via adenylate cyclase and phospholipase C. Stimulation of either pathway results in enhanced protein kinase activity and the ensuing alterations in ion channel phosphorylation. In the pituitary model system GH_3, stimulation by TRH or VIP acting respectively via phospholipase C or adenylate cyclase, causes biphasic changes in cytosolic Ca^{2+} transients due to electrical activity: a transient arrest due to inactivation of Ca^{2+} channels followed by a change in the frequency of action potentials. Both stimuli can change the amplitudes of Ca^{2+} transients. Thus, Ca^{2+} channel phosphorylation by either proteinkinases A or C produces the same repertoire of alterations in electrical activity. Receptor modulation of electrical activity is also obtained via K^+ channels, of which an important fraction is sensitive to cytosolic Ca^{2+} changes. During action potential firing, rapid (subsecond)localized oscillations in cytosolic Ca^{2+}, similar to those ocurring during receptor stimulated Ca^{2+} mobilization, may be observed. Thus, Ca^{2+} influx is directed towards discrete regions of the cells,the localization of which will determine K^+ channel regulation.

S13 3

ION CHANNELS AND RENIN SECRETION

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Opposite to typical secretory cells in which a rise of intracellular calcium initiates, facilitates or maintains secretion, calcium is thought to exert a negative control on renin secretion from renal juxtaglomerular (JG) cells. The mechanisms for this calcium "paradox" in JG cells is unknown. Recently the existence of calcium activated chloride channels in JG cells was found. In concert with voltage operated potassium channels these chloride channels probably mediate a calcium regulated volume control of JG cells. A rise of intracellular calcium would lead to cell shrinkage and vice versa. It is known from a number of experiments that renal JG cells behave as very sensitive osmometers. Increase of extracellular osmolality leading to cells shrinkage causes a prompt and potent inhibition of renin secretion. Conversely, a decrease of extracellular osmolality leads to an enhancement of renin secretion.

S13 4

REGULATION OF CALCIUM INFLUX BY SECOND MESSENGERS

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Simultaneous patch-clamp and fura-2 measurements were performed to study the mechanisms involved in second messenger-mediated calcium signals in rat peritoneal mast cells. We have identified three mechanisms that are activated following receptor stimulation and that may enhance secretion by maintaining elevated levels of $[Ca]_i$ due to calcium influx:

1) a voltage-insensitive cation channel of ~50 pS conductance is activated by external stimuli. It allows divalent cations to permeate according to the electrochemical gradient
2) a hyperpolarization-driven calcium influx activated by both external stimuli and by intracellularly applied IP_3. This calcium entry pathway must be highly calcium-specific, since it is paralleled by small whole-cell currents of 1-2 pA with no detectable single channel activity.
3) a chloride current activated by external stimuli and by intracellularly applied cAMP or high $[Ca]_i$. This current will clamp the membrane potential to negative values, thus providing driving force for calcium influx through the cation channels and the IP_3-dependent pathway

This novel combination of second messenger systems provides a flexible means to modulate calcium-dependent processes in cells that do not possess classical voltage-activated calcium channels.

S14

Symposium 14: "Cholesterol and Nutrition"

The Abstracts of this symposium appear elsewhere.

S15 1

BACTERIAL PHOTOSYNTHESIS - A LONGTERM CHALLENGE TO MICROBIOLOGISTS

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The concept of photosynthesis in green plants in the 19^{th} century. Discovery of bacteria responding to light and dark by Engelmann. Winogradskys concept of chemosynthesis and the purple sulfur bacteria. Molischs discovery of purple nonsulfur bacteria and his concept of photoassimilation of organic compounds. Buders reconciliation theory. Van Niels unequivocal demonstration of light and hydrogen donor-dependent CO_2 assimilation; his generalized theory of photosynthesis. The discoveries of the reductive pentose-P-cycle and of photophosphorylation. The role of light and the pathway of carbon. The fine structures and the differences of the cellular constituents serving photosynthesis in purple and green bacteria. Reaction centers and light-harvesting units.

S15 2

BACTERIAL ANTENNA COMPLEXES AND THEIR APOPROTEINS: STRUCTURAL HOMOLOGY AND VARIABILITY

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Species of the three families of purple bacteria (Rhodospirillaceae, Ectothiorhodospiraceae and Chromatiaceae) contain antenna complexes with a large spectral variability which may range in the near infra-red from around 800 nm to 1020 nm. The distinct spectral forms of the individual antenna complexes are the result of a specific (non-covalent) binding of BChl a or BChl b to antenna-specific apoproteins. The basic structural element has been elucidated as α/β-polypeptide heterodimers which form larger aggregates in vivo. The determination of amino acid sequences of a number of different bacterial antenna apoproteins represents now a starting point to formulate hypotheses about the structural-spectral relationships and suggest targets for site-directed mutagenesis experiments. In particular, the specific location of nucleophiles such as histidines, asparagines or lysines in the membrane-spanning domain, present at consensus distances of approx. 4 to 6 a.a. residues from the border line of the membrane, indicate their specific role in pigment binding. Similarly, many eucaryotic pigment-protein apoproteins such as the CCPP II polypeptide pair exhibit nucleophiles in equivalent positions, pointing to a conserved antenna-pigment organisation in procaryotic and eucaryotic organisms.

S15 3

Light-Harvesting Polypeptides as Models for the Study of Pigment-Protein Interaction

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Three types of light-harvesting complexes are found in phototrophic purple non-sulphur bacteria, B875, B800-850, B820, the number indicating the absorption maximum of the main infra-red absorption. In *Rhodospirillum rubrum* only a single light-harvesting complex, B875, is found, its simplicity makes it amenable to structural analysis of the mechanisms responsible for the highly efficient energy transfer in these complexes.

The functional chromophore is bacteriochlorophyll a (BChl a) which is bound to two non-identical polypeptides (α and β) with a stoichiometry of 2 mol BChl/mol αβ. In addition 1 mol of the carotenoid spirilloxanthin is bound per mol αβ. Both polypeptides have a molecular weight of approx. 6000 kD. The high efficiency of light-energy transfer is determined by the specific orientational ordering of the BChl chromophores given by the protein matrix. It has been shown that the complexes exist in different aggregational states and these states determine the extent of interaction between chromophores. This affects intensity and absorption maximum of the near infra-red transition (Q_y).

In vitro studies of the B875 complex from *R. rubrum* suggest that the assembly of the B875 occurs through several intermediate aggregational states each of which shows a characteristic absorption spectrum. Limited detergent solubilization of the B875 complex yields the intermediate B820 composed of 2 αβ dimers and 4 BChl molecules. Reconstitution of the B875 complex is achieved from B820 by dilution with detergent solubilized phospholipids. Reaggregation of the B820 leads to the formation of two-dimensional crystalline sheets.

The efficiency of light-energy transfer is regulated by protein phosphorylation. The B875 complex from *R. rubrum* is phosphorylated by a water-soluble protein kinase, the extent depending upon the level of reduction of the quinone pool and the cytochrome bc_1 complex.

S15 4

REGULATION OF PROTEIN SYNTHESIS IN CHLOROPLASTS

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In the algae Chlamydomonas reinhardii cell division and synthesis of individual proteins are induced by a diurnal light/dark regime. To study the regulation of protein synthesis in chloroplasts we compared the rate of synthesis of the 32 kDa herbicide-binding protein, the large subunit of the ribulose-bisphosphate carboxylase and the chloroplast elongation factor EF-Tu in whole cells during the cell cycle and also in isolated intact chloroplasts and in a chloroplast lysate. In different translation systems the rate of two studied proteins were influenced not in the same ratio. Furthermore, we were able to modify the rate of synthesis in intact chloroplasts by the addition of a cytoplasmic soluble fraction.
Simultaneously in isolated chloroplasts we determined the content and the distribution of the mRNAs between thylakoid-bound and free polysomes for these proteins. In the light period, when protein synthesis was highest, about 80% of the mRNA for membrane and soluble proteins were bound to thylakoids.

S15 5

NUCLEAR GENES REQUIRED FOR POST-TRANSCRIPTIONAL STEPS OF CHLOROPLAST GENE EXPRESSION IN CHLAMYDOMONAS.

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Two genomes contribute to the biogenesis of the chloroplast: while some polypeptides and RNAs are encoded in the chloroplast DNA, most polypeptides are encoded in the nucleus, translated in the cytoplasm, and then imported, processed and assembled in the organelle. We have studied the nuclear contributions to chloroplast gene expression by analyzing photosynthetic mutants of Chlamydomonas reinhardtii. Some of the nuclear mutants are defective because they fail to synthetize specific chloroplast-encoded components of the photosynthetic complexes. This dependence on the nuclear genome has intriguing properties: 1) the different nuclear factors are required specifically for the expression of single chloroplast genes, 2) they are required in post-transcriptional steps of gene expression, 3) a large number of nuclear genes are implied in the process. These features will be discussed in the light of examples such as *trans*-splicing, the stability of specific plastid mRNAs and the translation or stabilization of certain polypeptides.

Poster

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MOLECULAR DISSECTION AND FUNCTIONAL ANALYSIS OF THE AMYLOID β-PROTEIN PRECURSOR. J.-M. Roch, M. Sundsmo, G. M. Cole, L. Refolo*, N. Robakis*, T. Saitoh and J.-M. Matthieu+ (Sponsor). Dept. of Neurosciences, School of Medicine, University of California, San Diego, La Jolla, CA, USA, *Mt. Sinai School of Medicine, New York, NY, USA and +Laboratoire de Neurochimie, CHUV, Lausanne, Switzerland.

Amyloid β-protein precursor (ABPP) was shown to be a growth regulating molecule, as tested on a fibroblast cell line (A1), the normal growth of which depends on the presence of exogenous ABPP in the medium (Saitoh et al., Cell 58:615-622, 1989). In order to gain more knowledge about this phenomenon, as well as the processing of ABPP, we used molecular and cell biological approaches. Specifically, we engineered new plasmids that direct the expression of various regions of ABPP. A1 cells which usually produce extremely low levels of ABPP mRNA and ABPP were transfected with these different plasmids. Stable transfectants were selected by G418 resistance and several clones were isolated and characterized. In addition, ABPP was analyzed by immunoblotting in the medium, membrane and cytosolic fractions of cultured cells. The data indicate that ABPP is cleaved and secreted, leaving the C-terminal transmembrane fragment in the cell. We also present the evidence that ABPP undergoes degradation through a lysosomal pathway.

102

Resting Chondrocytes in Culture Survive Without Growth Factors, But are Sensitive to Oxygen Derived Radicals

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Chondrocytes in dense suspension culture in agarose survive in serum-free DME because they secrete an agent supporting their own viability. This activity resides in low molecular mass molecules and were replaced by sulfhydryl components, e.g cysteine or dithioerythritol, or by pyruvate. Catalase, an enzyme decomposing H_2O_2, protected the cells whereas superoxide dismutase had no effect. Therefore, chondrocytes in culture are sensitive to oxygen derived toxicity, probably in the form of hydroxyl radicals generated spontaneously in DME containing ascorbate and ferrous ions. Poly-ADP-ribosylation may be a step in the cascade of events triggered by the radicals.

For their survival, chondrocytes do not require stimulation by growth factors. They remain resting cells in fully defined, serum-free culture also low density. Proliferation and hypertrophy can be induced by serum factors and does not result from low cell density alone.

103

IN VIVO-MODULATION OF CHONDROCYTE ACTIVITIES BY IGFI AND hGH IN RAT GROWTH PLATE CARTILAGE

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The effects of IGFI and hGH in vivo on individual chondrocytes in growth plate cartilage were analyzed in hypophysectomyzed rats. Test substances were applied by osmotic minipumps over a period of 8 days. Growth rates, structural cell parameters and all kinetic data were obtained by fluorochrome labelling, incident light microscopy, light microscopic stereology and autoradiography. Mean structural cell parameters (such as linear dimensions, volume, net matrix production) of individual chondrocytes were influenced upon application of IGFI and hGH to a similar degree, both during proliferating and hypertrophic activity phases. However, in the presence of hGH, these cell activities were effected over a much shorter time period (-70%). - Linear cell production rates per cell column increased in parallel with the growth rates (IGFI: +200%; hGH: +430%). In the case of IGFI, this was effected solely by reduction of the mean cell cycle time (-60%); in the case of hGH, it was a combined effect brought about by a decrease in mean cell cycle times (-75%) and an increase in the linear dividing cell pool ("growth fraction"; by +30%).

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"MICROLIVER" OR TRIDIMENSIONAL CULTURE OF LIVER CELLS: BIOMATRIX EXPRESSION AND FUNCTIONAL ACTIVITIES
Juillerat, M., Kolodziejczyk, E., Vautravers, P., Coeytaux, S., Sierra, F. and Guigoz, Y., Nestlé Research Centre, Vers-chez-les Blanc, CH-1000 Lausanne 26.
Maintenance of tissue-specific functions in cultured hepatocytes appears to require direct cell-cell contact or biomatrix substratum. We have isolated the major liver cell types:sinusoidal cells (Kupffer, endothelial and fat-storing cells) by pronase treatment, density gradient and elutriation, and hepatocytes by collagenase treatment. The different cell types are then mixed in the proportion found in vivo and set in rotary culture in a defined medium. After 2-3 days in culture, round aggregates of 70-250 μm in diameter are formed. We have defined reproducible culture conditions in which the cell-aggregates express and secrete high level of albumin, while maintening a low level of expression and secretion of acute phase proteins, such as thiostatin. Cellular shape is maintained by endogenous deposition of extracellular matrix and intracellular formation of cytokeratins, and normal liver organisation is approximated. We are planning to use these "microliver" cultures, aggregate cultures of liver cells, for studies of nutrient metabolism, age-related changes in acute inflammatory response of the liver in vitro, as well as studies of xenobiotic metabolism.

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MOUSE MAMMARY GLAND INVOLUTION AS A MODEL FOR PROGRAMMED CELL DEATH

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Upon completion of lactation, the mouse mammary gland undergoes a dramatic tissue remodelling. Northern blot analysis of RNA extracted from mammary glands at different stages in involution shows that specific genes involved in metabolism (LDH and ODC), stress response (heat shock protein 70) and proteolysis (plasminogen activator and collagenase) are transiently expressed. This suggests that mammary gland involution, which results in death of more than 50% of the epithelial cells in the lactating organ, results from a specific program of gene expression.

We have developed an in vitro system for study of mammary epithelial cell differentiation. This system, which can be reduced to an epithelial cell clone and mesenchymally-derived extracellular matrix, differentiates when stimulated with lactogenic hormones. We are using this system to study the process of mammary gland involution after lactogenic hormone withdrawal.

106

REGULATION OF BETA-ADRENERGIC RESPONSIVENESS DURING ERYTHROID CELL DIFFERENTIATION

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Dimethylsulfoxide (DMSO) induces mouse erythroleukemia cells (MELC) to differentiate along the erythroid pathway. Within 26 h after induction of differentiation beta receptor density increased transiently from 3 to 11 fmol/10^6 cells, while receptor-coupled cAMP formation rose from 10 to 139 pmol/30 min. Between the 2nd and 5th day when the cells differentiated morphologically into normoblasts, receptor density decreased below the pre-induction level but receptor-coupled cyclase activity remained markedly elevated. Improved receptor-cyclase coupling probably resulted from parallel changes in membrane G-protein concentrations: While the Gi:Gs ratio in native cells was 7, a value of 0.5 was observed in differentiated cells. The early peak in beta-adrenergic sensitivity may be related to a transient stimulation of erythroblast proliferation by catecholamines. The change in G-protein expression points to a more general modulation of signal transduction during MELC differentiation.

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MAMMARY EPITHELIAL CELL SPECIFIC TYROSINE KINASES

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We are interested in the molecular characterization of novel epithelial cell specific tyrosine kinases especially those involved in differentiation of mammary tissues. As a model system we have two mouse mammary cell clones, one fibroblastic the other epithelial, which upon co-culture in vitro differentiate both morphologically and functionally. We are using two approaches; cDNA made from fibroblast epithelial and mixed differentiated cultures has been amplified using the PCR and primers corresponding to highly conserved regions of the tyrosine kinase domain. We are cloning the amplified materia l and are looking for specific clones by differential screening. Secondly, we have raised specific antibodies against peptides derived from highly conserved regions of the tyrosine kinase domain. These antibodies react with several known kinases. We are characterizing epithelial cell tyrosine protein kinases particularly those involved in differentiation.

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THE RESPONSE TO VASCULAR INJURY INVOLVES INCREASED EXPRESSION OF SEVERAL GROWTH FACTORS AND ONCOGENES

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Proliferation of smooth muscle cells (SMC) is part of the pathology of arteriosclerosis. Balloon catheterization of the rat aorta provides an opportunity to examine gene expression in vivo during the induction of SMC proliferation in response to vascular injury. At serial times for 24 hours(h) after injury we extracted total RNA from placebo or balloon injured rats and determined levels of mRNA by Northern blot. PDGF A mRNA increased 2 h after injury, peaked (5 fold) at 6 to 9 h, and decreased by 24 h. PDGF B mRNA did not change. TGF-β mRNA increased slightly sooner after injury and peaked at 6 to 8 h. Thrombospondin mRNA, very low until 30 min after injury, increased rapidly by 3 h, and remained high for up to 24 h. c-fos and c-myc mRNA levels peaked at 30 min and 2 h after injury, respectively. Similar changes in mRNA levels for these factors were observed in vitro after addition of 10% serum to rat aortic SMC previously growth-arrested in serum-free media.

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BONE AND CARTILAGE FORMATION BY IMMORTALIZED CELL LINES OF THE OSTEOBLASTIC PHENOTYPE

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The clonal cell population CRP10/30, expressing the markers of a differentiated osteoblast, such as alkaline phosphatase, PTH- and PGE_2-induced cAMP production, synthesis of collagen type I and osteocalcin, has been immortalized by the introduction of the avian OK10 v-myc proto-oncogene. Two stable cell lines, expressing different phenotypes, have been chosen for further studies. Both lines produce osteocalcin and are therefore representative for differentiated osteoblastic cells. When cultured in vivo in diffusion chambers, both lines proved to be osteogenic. However, besides bone, cartilage, characterized morphologically and by the expression of collagen type II, and fibrous tissue were fromed. These results demonstrate that differentiated cells of the osteoblastic lineage still retain a multipotential differentiation capability when cultured in vivo.

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MOUSE OOCYTE MATURATION, FERTILIZATION AND EMBRYO CULTIVATION IN VITRO IN INBRED STRAINS WITH STAGE SPECIFIC BLOCKS DURING EARLY EMBRYOGENESIS.

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Recently developed in vitro techniques for oocyte maturation, fertilization and embryo culture enabled us to study developmental capacities of oocytes of mutant mouse strains with 2-cell (DBA)- OR morula/blastocyst (Balb/c) stage block during embryogenesis. We used germinal vesicle stage oocyte cumulus cell complexes from (C57BL/6J X SJL) F1 mice as controls and male animals from the same strain as donors of sperm. For maturation, we applied Waymouth MB 752/1 medium, for fertilization Whitten's- and for embryo culture CZB-medium. After maturation of oocytes under standardized conditions, we could not detect the above stage specific blocks during development up to blastocyst, suggesting beneficial effects of the in vitro system.

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LACK OF GRANULOCYTE COLONY-STIMULATING FACTOR (G-CSF) PRODUCTION BY CALVARIA OF THE OSTEOPETROTIC OP/OP MOUSE

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The murine osteopetrotic variant op/op is characterized by impaired bone resorption and low number of osteoclasts. We have previously shown that cells from calvaria and other connective tissue cells of the affected (op/op) mice, in contrast to the phenotypically normal littermates (+/?), do not produce macrophage (M)-CSF. We now investigated whetner other CSFs might also be lacking. Calvaria of 10 days old mice were incubated in BGJ_b medium in the presence of 100 µg/ml LPS. Hemopoietic growth activity in the conditioned medium (CM) was separated on Phenyl-Sepharose CL-4B columns into granulocyte-macrophage (GM-) and G-CSF. These activities were identified and measured by assessing the effect on the proliferation of different cell lines, by colony assay in semisolid media and by inhibiting GM-CSF with a specific antiserum. In contrast to CM from +/? calvaria, no G-CSF was found in CM from op/op calvaria. However, in CM of lungs no difference between +/? and op/op was observed.G-CSF is produced by macrophages (MØ). Since the resident MØ are absent in bone and bone marrow in tne op/op mouse, the absence of G-CSF may be secondary to tne lack of M-CSF. G-CSF may in turn play a direct role in the osteoclastogenesis.

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ESTABLISHMENT AND GERM LINE INTEGRATION OF C57BL/6 EMBRYONIC STEM CELLS

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Murine embryonic stem (ES) cell lines have been derived from outgrown C57BL/6 blastocysts. Selected male cell lines have been tested for karyotype stability, in vitro and in vivo developmental potential. After injection into BALB/c host blastocysts, one of these lines (BL III) has participated with particular high frequency in the formation of chimaeras. Breeding tests have revealed a germ line contribution of the BL III ES cells in about 50% of the chimaeras.
This C57BL/6 ES cell line is currently used for gene targeting experiments.

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CLONING AND EXPRESSION OF AN RNASE A HOMOLOGUE FROM MURINE SPLEEN

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Despite the potential importance of homologues of the pancreatic (secretory) RNases in control of cell growth, little is known about the genes for non-secreted members of this family. To identify potential regulatory homologues of RNase A, we have started screening a λgt11 library made from murine spleen poly-A$^+$ RNA using an oligonucleotide based on the bovine DNA sequence coding for the extremely conserved amino acids 40-50 of RNase A. One cDNA clone from an abundant messenger is about 750 n long, and homologous to the rat and murine pancreatic RNase genes. We will use this clone to screen a human cell line (Colo) cDNA library. We are testing bacterial expression systems to produce enough protein to determine its enzymatic activity and possible interaction with lymphokines that inhibit cell growth.

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SYNCHRONIZATION OF CHEMICALLY TRANSFORMED FIBROBLASTS WITH NOCODAZOLE

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A rat fibroblast cell line (T14c) obtained by exposure to the carcinogen MNNG expresses high amounts of the tumor specific oncomodulin, a parvalbumin-like protein. Calmodulin, another member of the family of high affinity Ca^{2+}-binding proteins, is essential for cell cycle progression. Our aim is to investigate if oncomodulin could play a similar role in tumor cells. T14c cells were synchronized by incubation for 12 hours with 0,4 ug/ml nocodazole, a rapidly reversible microtubule inhibitor, resulting in mitotic arrest of 50% of the cell population. A yield of 80% mitotic cells was obtained upon harvesting. On release into fresh medium T14c cells left the mitotic phase within 2 hours to reenter mitosis synchronously about 24 hours later, a period corresponding to the doubling time for unsynchronized T14c cultures. Oncomodulin and calmodulin transcript levels will be analyzed in cells at different stages of the cell cycle by the use of specific cDNA probes.

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STRUCTURE AND PROMOTER ACTIVITY OF RAT ENDOGENOUS LTRs

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Oncomodulin is a tumorspecific Ca^{2+}-binding protein, structurally related to parvalbumin. A computer search showed a high similarity between the 5' cDNA leader sequence of oncomodulin and part of a Syrian hamster IAP LTR. Using oncomodulin cDNA as a probe we screened a rat lambda genomic library and isolated 28 independent recombinants. A subclone of one of these isolates (H12) contains a LTR-like sequence with all the typical features of a retroviral promoter and 93 % sequence identity to the oncomodulin promoter. Other rat IAP LTR clones were partially sequenced. They represent a family of closely related endogenous LTRs differing only slightly from the oncomodulin promoter. To investigate the promoter activity of our cloned LTRs and the oncomodulin LTR obtained by PCR, we ligated several LTRs into a vector carrying the human growth hormone reporter gene. These constructs will be transfected into chemically transformed rat fibroblasts (T14c) which have been shown to express oncomodulin. Normal fibroblasts that do not synthesize oncomodulin serve as negative controls. These expression studies using naturally mutated LTRs should allow to delineate cis-acting elements important for gene activation.

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PHENOTYPIC DIVERSITY IN A SERIES OF CELL-CYCLE MUTANTS DERIVED FROM A MURINE MASTOCYTOMA

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A series of heat-sensitive cell-cycle mutants belonging to 6 different complementation groups (CGs) was obtained from the murine P-815-X2 cell line by mutagenization, appropriate selection and isolation of subclones. Of 20 mutants tested, all were arrested in G1 phase upon transfer to the nonpermissive temperature of 39.5°C. Arrest in G1 was, however, rapid in 4 of the 6 CGs only. Cells of one clone slowly changed to tetraploidy at 39.5°C. Typical differences in cell survival at 39.5°C were observed even between clones of the same CG. Of some clones, cell numbers remained nearly constant at 39.5°C for at least 20 days. One CG was characterized by a marked increase in cell size at 39.5°C, and some clones of at least 2 CGs underwent morphological mast-cell differentiation with formation of metachromatic granules at 39.5°C.

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RADIOPROTECTION OF CULTURED CELLS BY PREINCUBATION IN MEDIUM CONTAINING DEUTERIUM OXIDE

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Pretreatment of mice with deuterium oxide (D_2O) protects mice against lethal effects of X rays, whereas X-irradiation of cultured cells in D_2O-containing medium was previously found to cause a decrease in cell survival. Therefore, the effects of a four-days preincubation in medium containing 20% D_2O were tested. Cells of a heat-sensitive murine cell-cycle mutant (21-Tb) cultured in normal or D_2O-containing medium, and proliferating at 33°C or reversibly arrested in G1 phase at 39.5°C, were exposed to single X-ray doses of 0 - 10 Gy, resuspended in normal medium, and survival was determined by the capacity of cells to form colonies. Preincubation in 20% D_2O resulted in a radioprotective effect for both proliferating and arrested cells particularly at higher X-ray doses, while no evidence for an increase in postirradiative repair capacity was obtained.

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POTENT MITOGEN IN SERUM-FREE MEDIUM OF PRIMARY CULTURES OF CHICKEN EMBRYO FIBROBLASTS

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We have found that serum-free conditioned medium (SFCM) of confluent cultures of chicken embryo fibroblasts (CEF) contains a strong mitogenic activity which promotes growth of NIH/3T3 cells and of secondary cultures of CEF. Half-maximum stimulation was obtained with 20% of initial SFCM. The maximum mitogenic activity was about 60% of that of 10% fetal calf serum (FCS) if the cells were counted two days after stimulation. The stimulation of thymidine incorporation in 3T3 cells was equal to that achieved with 10% FCS and reached its maximum 16 hours after stimulation. The mitogenic activity elutes at high M_r (6 to 20 x 10^4) from a gelfiltration column and is trypsin- and thiol-insensitive. Treatment with 0.1% trifluoroacetic acid and 20% acetonitrile as well as the addition of detergent renders the mitogen to a low-molecular, biologically less active form. Its physico-chemical properties suggest that the stimulator is not identical with one of the common growth factors like IGF, FGF, EGF, TGF-β or PDGF. Present efforts focus on its further purification in order to find out whether it represents a novel type of growth factor.

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CELL CYCLE DEPENDENT PHOSPHORYLATION OF THE CHICKEN CDC2 KINASE

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The cdc2 kinase is part of a regulatory network controlling the eukaryotic cell cycle. Recently, entry of cells into mitosis was shown to be accompanied by dephosphorylation of the catalytic subunit ($p34^{cdc2}$) of this kinase. To examine the cell cycle dependency of phosphorylation of chicken $p34^{cdc2}$, we determined its phophoaminoacid and phophopeptide composition at various stages of the cell cycle. In exponentially growing cells $p34^{cdc2}$ was found to contain phospho-serine (P-S), threonine (P-T) and tyrosine (P-Y). Two-dimensional tryptic peptide maps of P^{32}-labeled $p34^{cdc2}$ revealed three major phosphopeptides. Two peptides contained both P-T and P-Y, whereas the third contained only P-S. Upon entry of cells into mitosis, $p34^{cdc2}$ was found to be dephophorylated on both T and Y sites, but, remarkably, P-S remained. Rephosphorylation of $p34^{cdc2}$ on both T and Y sites occurred during G1 phase of the cell cycle.

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CELL CYCLE REGULATION: CLONING AND CHARACTERIZATION OF CHICKEN CYCLINS

P. Gallant and E.A. Nigg, ISREC, CH-1066 Epalinges

Cyclins were originally discovered in marine invertebrates as proteins that are synthesized continuously throughout the cell cycle and destroyed abruptly at the metaphase-anaphase transition. A critical role of these proteins in cell cycle regulation is indicated by genetic analyses in yeasts. Moreover, microinjection experiments in frog oocytes demonstrate that cyclins are required for entry into M-phase, and, conversely, that their destruction is necessary for exit from M-phase. At present, comparatively little is known about the regulation and detailed mode of action of cyclin proteins in higher vertebrates. In order to address these issues, we have cloned chicken cyclin(s), using a frog cyclin B probe (kindly provided by Dr. T. Hunt, Cambridge) for low stringency hybridization. A characterization of the structure and expression of chicken cyclin(s) will be presented.

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IN VITRO DISASSEMBLY OF THE NUCLEAR LAMINA AND M-PHASE SPECIFIC PHOSPHORYLATION OF LAMINS BY HIGHLY PURIFIED CDC2 KINASE.

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The nuclear lamina is an intermediate filament type network underlying the inner nuclear membrane. Phosphorylation of lamin proteins has been implicated in causing lamina disassembly during meiotic and mitotic M-phase, but the M-phase specific lamin kinase has not been identified. Here we show that the cdc2 kinase, a major element implicated in controlling the eukaryotic cell cycle, phosphorylates chicken B-type lamins in vitro on sites that are specifically phosphorylated during M-phase in vivo. Concomittantly, cdc2 kinase is capable of inducing lamina depolymerization upon incubation with isolated nuclei. Conversely, although both protein kinases A and C readily phosphorylate lamins, neither M-phase site phosphorylation nor lamina disassembly can be induced by these enzymes. One of the target sites of cdc2 kinase is identified as a motif (SPTR) conserved in the N-terminal domain of all lamin proteins. These results lead us to propose that mitotic disassembly of the nuclear lamina results from direct phosphorylation of lamins by cdc2 kinase.

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A CELL CULTURE MODEL TO STUDY RADIATION EFFECTS OF HOT PARTICLES

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Hot particles are highly radioactive particles made up of insoluble α-, β- and γ-emitters. Being small enough to become airborne, they are easily inhaled and deposited in the lung. The radiation dose becomes very high for cells directly adjacent to the particle and decreases very fast within a few cell diameters. One critical effect for the affected tissue might be local growth stimulation in response to the emerging microlesion leading to cell proliferation in a sublethal radiation field and possibly to transformation and loss of growth control.
A small neutron-activated yttrium-wire serves as a source for an inhomogeneous radiation field. Yttrium-90 is a strong beta-emitter. The wire is placed below the cell monolayer, and the cells are exposed during 24 hours. The dose decreases from several hundred gray per hour above the wire to one gray within a few millimeters. Survival was correlated with the distance and proliferation induction was investigated by autoradiography.

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REDUCED CALCIUM REQUIREMENT FOR GROWTH IS CORRELATED WITH INTRACELLULAR CALCIUM STORES IN NORMAL AND SV40-TRANSFORMED 3T3 CELLS.

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We used SV40 transformation of NIH and Swiss 3T3 cells to study calcium requirements for growth. Neither untransformed nor SV 40-transformed Swiss 3T3 cells proliferate well in media with 50 µM calcium. With the Ca^{++}-sensitive dye Fura-2 we could demonstrate that these cells had depleted intracellular calcium stores. The depletion of the stores led to a much-decreased intracellular calcium transient in response to serum and ionophore. In contrast, SV40-transformed NIH 3T3 cells proliferated in the 50 µM medium and maintained their stores. Furthermore, untransformed NIH 3T3 cells could be conditioned to grow in the low-calcium medium and, after conditioning, maintained their calcium stores and serum response in low calcium media. We also observed differences in basal calcium levels in transformed and untransformed cells. Our experiments indicate however, that it is the ability of the cells to maintain adequate calcium stores in low calcium media that corelates with a full serum response and the ability to proliferate, rather than alteration in resting calcium levels per se.

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CHEMICALLY INDUCED G_0-G_1-CELL CYCLE PHASE SHIFTS IN CULTURED RAT HEPATOCYTES ANALYZED BY IMAGE ANALYSIS.

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Chemicals which interfere with cellular growth processes might be potential nongenotoxic carcinogens or tumor promoters. The decondensation of the nuclei which occurs during the shift from the G_0 to the G_1 cell-cycle phase can be detected with the quinacrine-dihydrochloride staining method and examined by image analysis. Hepatocytes in serum free cultures were exposed for 3-4 days to an unspecific growth stimulus (10% FCS), to a differentiation inducing agent (dimethylsulfoxide) and to three well known rodent liver tumor promoters (phenobarbital, cyproterone-acetate, thioacetamide). Upon analysis of hepatocyte monolayers or isolated nuclei, each of the test chemicals induced a specific pattern of nuclei decondensations. The alterations seen were dose dependent, exposure time dependent and specific for individual ploidy subpopulations. This cell cycle phase shift could be useful for the detection of agents which interfere with differentiation (aging) and growth of hepatocytes (nongenotoxic carcinogens or liver tumor promoters). (Schweiz. Krebsliga 389.88.1)

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IDENTIFICATION AND PURIFICATION OF PROTEIN(S) THAT BIND TO THE HISTONE H4 ARS CONSENSUS IN YEAST

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An autonomously replicating element (ARS), is located downstream to a yeast histone H4 gene. Several DNA binding activities can be identified by various footprinting techniques in this region.

Hydroxyl radical footprinting revealed a protection that covers the ARS consensus sequence. The 5' border of this protection is identical with the endpoint of deletions that still retain ARS activity (Smith and Bouton, 1986).

Specific binding was also detected in gel-retardation assays using nuclear extracts or soluble scaffold extracts, with an oligonucleotide representing the protected ARS sequence as a probe. Binding affinity was considerably higher for the T-rich strand alone than for the duplex.

Affinity chromatography and UV-crosslinking identified four proteins between 70 and 55kD. Current work addresses the identity of these proteins, their affinity for other ARSs, and the possible role of these factors in initiation of DNA replication.

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REPLICABILITY OF MODIFIED RNA TEMPLATES BY BACTERIOPHAGE Qß REPLICASE

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Full-length bacteriophage Qß cDNA (4217 bp) as well as variants with internal deletions or insertions (kanamycin- or tetracyclin-resistance genes) were cloned in heat-inducible cells downstream of the λPL promoter, with the aim to establish stable RNA replicons. Provided that the sequence encoding the phage replicase was kept intact, Qß-specific RNAs of both polarities accumulated in transfected cells kept at low temperature; however, their size was predominantly small. Heat-induction resulted in the transient formation of replicating RNAs of the correct size, which in turn were soon almost completely replaced by the small RNAs, presumably consisting of spontaneously formed deletion mutants outcompeting the long transcripts. To study in vitro the details of template function of modified Qß RNAs of both polarities, we constructed vectors based on modified bluescript plasmids affording production of transcripts with defined termini (natural or changed at will). Surprisingly, replicase reactions showed that all the terminally and internally modified Qß RNAs were copied by the enzyme, although with generally lower and different efficiencies. Not only the initiation of product RNA chains depends on the template structure, but also the efficiency of chain elongation.

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REPLICATION ORIGIN ACTIVITY AND NUCLEAR SCAFFOLD ATTACHMENT

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We have previously reported that genomic ARS elements are attached to the nuclear scaffold in the budding yeast *Saccharomyces cerevisiae*. More recently, we have found analogous interactions in the fission yeast *Schizosaccharomyces pombe*. Scaffold attached regions (SARs) from animal cells share some sequence similarities with these regions in yeast, suggesting possible similarities in function. To address this question, we have studied SAR elements from Drosophila melanogaster (dSARs) for their ARS activity and scaffold attachment in the two yeast species. The four dSARs tested can bind in vitro to scaffolds from both yeasts. Three dSARs have ARS activity in *S. pombe*, while two are ARSs in *S. cerevisiae*. Fragments flanking the dSARs neither promote replication, nor bind to the yeast scaffolds. We chose to further investigate the dSAR from the *Drosophila* ftz locus, which shows ARS and scaffold binding activities in both yeast species, by deletion and subcloning experiments (in collaboration with L. Pick and W. Gehring, Basel). We have characterized the minimal sequence elements required for replication and scaffold association in *Saccharomyces cerevisiae*. Multiple elements appear to be involved in both functions.

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BETALACTAM ANTIBIOTIC DERIVATIVE INHIBITING REVERSE TRANSCRIPTASE AND CELLULAR DNA POLYMERASES

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Proliferating eukaryotic cells are affected by degradation products of betalactam antibiotics (Neftel, K. A. and Hübscher, U. Antimicrob. Agents Chemother. 31, 1657-1661, 1987). We have purified the major degradation product of a cephalosporine betalactam antibiotic to homogeneity. After the betalactam-ring has been opened in aquoeus solution or by enzymatic treatment with betalactamase the product was purified by chromatography on a silicagel-column and by HPLC. This product was able to inhibit the replicative DNA-polymerases α, δ and γ of HeLa-cells and moreover reverse transcriptases from human immunodeficiency (HIV) and feline immunodeficiency (FIV) virus. Data on the mode of inhibition will be presented.In feline lymphocyte cell culture we could show that in the presence of this derivative no FIV infection occured under conditions where host cell growth was unaffected.We discuss the usefulness of this FIV cell culture system on a model to study HIV-infection.

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ANTI-CYTOSKELETAL DRUGS AFFECT PLASMA MEMBRANE DISTRIBUTION OF BRAIN mRNA INDUCED Na^+-CHANNELS IN XENOPUS OOCYTES

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We have previously reported accumulation at the animal pole (non-random distribution) of newly expressed neuronal ion channels in the plasma membrane of Xenopus oocytes after microinjection with poly A^+ mRNA. We now have studied the effect of cytoskeletal toxins (10 µM colchicine or 2µM cytochalasin D) on this distribution of voltage dependent Na^+-channels. When the toxins were present during the entire expression period, we found random insertion into the surface membrane, while the extent of functional expression remained unaffected. The randomization of the Na^+-channel insertion by colchicine is dose-dependent (Ka=0,5µM). The control substance ß-lumicolchicine had no effect. Taxol inhibited the functional expression in a dose-dependent manner (Ki=1,5µM). Our results suggest that cytoskeletal elements play an important role in the sorting of membrane proteins to different surface membrane domains in the Xenopus oocyte. Thus, Xenopus oocytes may provide a model system for the study of plasma membrane protein sorting in polarized cells.

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DOES BIOTIN INFLUENCE CYTOKERATIN EXPRESSION ?

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The vitamin, biotin, is an essential cofactor in cellular transcarboxylation reactions. Clinical studies have shown that biotin administration improves hoof quality in farm animals and horses, and that experimental biotin deficiency leads to parakeratosis in laboratory animals. In order to study the molecular mechanism underlying these observations, we decided to investigate the effect of biotin on the expression of cytokeratin proteins in the kerationcyte cell line, HaCaT. Using avidin to eliminate biotin from culture medium, we found that the pattern of cytokeratins expressed was different in biotin-depleted versus -repleted cultures, and that the pattern also depended on the proliferation state of the cells. The present data suggest that the expression of a cytokeratin protein specific for well differentiated keratinocytes is biotin-dependent.

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LOCALIZATION OF ACTIN AND CHARACTERIZATION OF ITS ISOFORMS IN THE HYPHAE OF *NEUROSPORA CRASSA*

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We have localized and characterized the actin isoforms of *N. crassa* in order to unravel their function during growth and development. The distribution of actin in the fungus *N. crassa* was examined by FITC-phalloidin staining of formaldehyde-fixed hyphae. FITC-phalloidin stained actin was found to be mainly concentrated in the hyphal tips in which it formed uniform cap lying essentially in the periphery of the hyphae. The subapical actin was visualized as spots. Their number varied in different hyphae but they were uniformly distributed along the length of the germ tubes outgrown from conidia. Three actin isoforms (with predominant β-isotype) were revealed by two dimensional gel electrophoresis. Apical actin could be involved in hyphal morphogenesis and in organelle motility.

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EFFECT OF PROTEIN KINASE ACTIVATION AND INHIBITION ON THE ASSOCIATION OF α-ACTININ WITH THE NEUTROPHIL CYTOSKELETON

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Upon activation of neutrophils by chemotactic agents, a rapid and dramatic increase in the amount of cytoskeleton-associated actin occurs. We have found, that this increase is paralleled by a significant increase in the association of the actin cross-linking protein α-actinin with the neutrophil cytoskeleton (V.Niggli and V. Jenni, Eur. J.Cell Biol.49, 366-372, 1989). Other agents, which are not chemotactic, such as activators of protein kinase C, and the protein kinase inhibitor staurosporine also increase the amount of cytoskeletal actin. We have now found, that this increase is paralleled, for both staurosporine and phorbol ester, by an increase in cytoskeletal α-actinin. Thus, non-receptor-linked activation of neutrophils affects the organization of both α-actinin and actin.

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A high molecular weight nuclear matrix protein shares an antigenic determinant with NF200K

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The nuclear matrix of eucaryotic cells, after extraction of DNA, RNA and soluble nuclear proteins, is composed of (I) the three lamins A,B and C (M_r 72,68, and 62 kD), which form a meshwork of intermediate-type filaments, lining the nucleoplasmic surface, (II) the nucleolus and (III) additional intranuclear proteins. In a human glioma cell lineage (M617) we found a HMW-polypeptide (M_r =195 kD; determined by measurement of relative mobility R_f in SDS-PAGE), which shares an epitope with the neurofilament constituent polypeptide NF200K. A patchy staining in nuclei of interphase cells are observed, whereas the protein in mitotic cells is diffusely spread throughout the cytoplasm. In situ fractionation study of the cells with NP40, high salt extraction and DNase I treatment, indicate, that the protein is a nuclear matrix protein. In vitro dephosphorylation of the polypeptide gave evidence, that it belongs to the highly phosphorylated proteins. In contrast to the lamins, this protein seems to be phosphorylated during interphase. A binding to the chromatin proteins with much lower M_r is described (J.Neurochem. 44: 149-154 (1985). (Supported by EMDO-Stiftung and Hartmann-Mueller-Stiftung, Zurich)

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FIBROBLASTS EXPRESS α-SMOOTH MUSCLE ACTIN DURING WOUND HEALING

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Previous studies have shown that in various fibrocontractive diseases, myofibroblasts express α-smooth muscle actin (α-SM actin) and/or desmin, both of these proteins being smooth muscle differentiation markers. In the present study we have examined the expression of these markers during healing of a skin wound in rats. Electron microscopy and immunohistochemistry at the light and electron microscopic level using a monoclonal antibody specific for α-smooth muscle actin (anti-α-SM-1) were performed. The surface area of the wound decreased linearly during the first 12 days the rate then slowed; at 20 days the wound was closed and re-epithelialized. Anti-α-SM-1 positivity was seen from day 6 when it was weak and focally distributed in fibroblasts. From 12 to 15 days anti-α-SM-1 positivity was strong in a large proportion of fibroblasts in the wound. From 20 to 30 days there was a decrease in expression and at 30 days only blood vessels were anti-α-SM-1 positive. From 20 to 30 days we observed the appearance of apoptotic fibroblasts. We believe that this may represent a route for the loss of myofibroblasts which occurs as the wound heals to leave a relatively acellular scar. In conclusion, myofibroblasts in a normally healing rat wound, transiently express α-SM actin, a marker of smooth muscle cell differentiation. This suggests that the persistent expression of this marker in fibrocontractive diseases may represent a deregulation of factors which stimulate α-SM actin expression during normal healing.

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IN VITRO REGULATION OF ARTERIAL SMOOTH MUSCLE CELLS (SMC) GROWTH AND PHENOTYPIC MODIFICATION BY HEPARIN.

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Rat aortic SMC placed in culture in the presence of 10% foetal calf serum show a typical cytoskeletal remodeling characterized by a switch in the pattern of actin isoform expression with the appearence of a β-isoform predominance, compared to SMC freshly isolated from the rat aortic media, which show a predominance of the α-smooth muscle (SM) isoform (Skalli et al., J Submicrosc Cytol 18:481, 1986). Previous studies have shown that heparin inhibits arterial SMC proliferation in vivo (Clowes and Karnovsky, Nature 265:625, 1977) and in vitro (Hoover et al., Circ Res 47:578, 1980). We have investigated the effect of heparin on actin isoform expression in cultured SMC. Western blots with a specific antibody (Skalli et al., J Cell Biol 103:2787, 1986) and two-dimensional gel electrophoresis demonstrated an increase in the expression of α-SM actin in the presence of heparin (100μg/ml, Sigma Co). Northern blots of total RNA extracts with an α-SM actin mRNA specific probe (Kocher and Gabbiani, Differentiation 34:201, 1987) indicated that heparin increases the expression of α-SM actin mRNA. Elucidating the action of heparin on SMC actin expression may contribute to a better understanding of SMC phenotypic modifications during atheromatosis.

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B-creatine kinase is expressed, like MyoD, in determined myogenic cells of the myotome.

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Early stages of differentiating myoblasts accumulate increasing amounts of B-CK and only after the induction of the M-CK gene B-CK expression is reduced. No M-CK nor Mi-CK mRNA was detectable in young embryos, however, there are significant amounts of B-CK transcripts. Creatine kinase expression was investigated in embryos at early stages of development (stages 12 and older; Hamburger & Hamilton) by *in situ* hybridization and the accumulation of the protein by immuno-histochemistry methods. The earliest expression of B-CK m-RNA was observed in the neural tube of stage 12, while no distinct accumulation of B-CK protein was detected. In more mature embryos, there was an additional hybridization in the middle part of the somites, which was identified in the following stages as the myotome. The B-CK protein was found at later stages (from stage 16 on) indicating that translational control may be active. Other probes are being utilized, like the CMD1-probe, a chicken MyoD homologue, and alpha-vascular actin and alpha-cardiac actin, two probes for early muscle differentiation and M-CK also typical for terminally differentiated muscle. We conclude that B-CK is expressed very early in myogenic cells and may be a marker for the presumptive myoblast and the myoblast itself. Furthermore is seems quite clear, at least in the chicken embryo, that the expression of MyoD like transcripts does not entail the switch to M-CK expression.

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Exploration of cytoarchitecture by universal epitope tagging.

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The building blocks of the cells architecture are composed of different proteins which often belong to isoprotein families consisting of members with similar protein sequences. In order to elucidate their structural and functional relationsships it was of major importance to develop a system that would allow to follow each of the components within the normal cellular environment. By site directed mutagenesis of the cDNA encoding the cytoskeletal protein of choice, an oligonucleotide was introduced coding for a defined foreign epitope against which antibodies are available. We report that the tagging of the chicken myosin light chain 1f with the C-terminal undecapepetide of the VSV G protein allows to precisely localize, with an anti-tag antibody, the protein expressed in cultured cells. The transfer of the engineered expression vector was achieved by a variety of methods including transient and stable transformation as well as nuclear microinjection. Tagged proteins can be expressed in a wide variety of cells, including non-myogenic human (HeLa) and myogenic mouse cell (C2) lines, chicken embryonic fibroblasts (CEF) as well as chicken primary heart and skeletal muscle cells. The tagged proteins are correctly sorted within the cytoplasm of the manipulated cells and show incorporation into the A-band of myofibrils. The nature of the tagged proteins will be further manipulated by site directed mutagenesis which will lead to an understandig of the structural requirements of functional building units within the cytoarchitecture.

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METABOLISM OF TESTOSTERONE IN THREE PROSTATIC CELL LINES.

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In the prostate, testosterone (T) is rapidly converted to dihydrotestosterone (DHT), the most potent androgen in this tissue, which can be further metabolized to (3α, 3β) 5α-androstandiol. To better understand T metabolism in prostatic cancer cells, we have compared three prostatic cell lines (DU-145, PC3, and LNCaP) with respect to their ability to metabolize [^{14}C]T. T and metabolites were analyzed using TLC techniques and a Berthold plate reader. DU-145 cells contain significant amounts of 5α-reductase and DHT is the major metabolite formed. This cell line also contains 3β-hydroxysteroid dehydrogenase (3β-HSDH) and 17β-hydroxysteroid dehydrogenase (17β-HSDH) activity. In PC3 cells, however, essentially no DHT was detected. Incubation of PC3 cells with [^{14}C] DHT revealed high concentrations of 17β-HSDH which converts DHT to 5α-androstandione suggesting that DHT might be formed from T but immediately converted. The majority of T in PC3 cells is converted to androstenedione. LNCaP cells contain a minimal amount of 5α-reductase activity and higher amounts of 3β-HSDH and 17β-HSDH activity. It is interesting that only DU-145 cells produced significant amounts of DHT, the major androgen in the prostate, while PC3 and LNCaP cells produce only minimal amounts of this androgen. These results show that prostatic cell lines markedly differ in their pattern of T metabolism.

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Urokinase-type plasminogen activator mRNA in implanting murine embryos

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Urokinase-type of plasminogen activator (u-PA) is produced by a variety of cell types endowed with migrating properties and involved in processes of tissue disruption. For instance, trophoblasts display a migrating and invasive phenotype in order to achieve embryo implantation, and these cells have been shown to produce u-PA in vitro. To assess the postulated participation of u-PA in trophoblastic invasiveness in-vivo, we performed in-situ hybridization studies on cryostat tissue sections of 5 to 10 days-old mouse embryos, using a specific murine u-PA ^{3}H-labelled cRNA probe. In 5 days-old embryos, moderate amounts of u-PA mRNA were detected in ectoplacental and peripheral flattened trophoblastic cells. In 6 days-old embryos, u-PA mRNA was abundant in trophoblastic giant cells invading the uterine wall, whereas in 7 days-old embryos, it was found predominantly in the trophoblastic cells whithin the uterine stroma. By the 8th day, in addition to a restricted number of peripheral giant trophoblastic cells, the ectoplacental glycogen cells differentiating into trophospongium displayed an intense signal. These results establish the in-vivo synthesis of u-PA by trophoblastic cells.The temporo-spatial pattern of u-PA mRNA distribution observed is compatible with an role for u-PA in the extra-cellular proteolysis accompanying embryo implantation.

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THE ABUNDANT AVIAN SMOOTH MUSCLE ($\alpha_1\beta_1$) INTEGRIN IS A COLLAGEN IV RECEPTOR

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Integrins are a family of membrane glycoproteins that link the cytoskeleton with the extracellular matrix. This structural property makes them an interesting object for biophysical investigations. So far only one integrin, derived from chick gizzard, has been purified in large enough amounts to allow such studies (J. Biol. Chem. **262**, 17189-17199). Immunological data showed that the ß chain is the chick β_1 chain, related to the β_1 chain of the mammalian VLA (very late activation antigen) subfamily of integrins. We determined the N-terminal protein sequence of the α chain and found a strong homology to the VLA-1 α_1 chain, which is known to act as a receptor for collagen IV. Primary embryonic chick gizzard cells express the $\alpha_1\beta_1$ integrin and attach to collagen IV. This attachment can be completely inhibited by polyclonal antibodies to the chick integrin α_1 chain. Furthermore the isolated receptor in detergent solution binds to collagen IV in ELISA style assays. The identification of the ligand for this integrin, which can be extracted in mg amounts, will make it possible to characterize the molecular interaction between collagen IV and its cellular receptor by biochemical and biophysical means. (Supported by NF 31-8826.86.)

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DOES THE u-PA RECEPTOR PLAY A ROLE IN THE INVASIVE/METASTATIC PHENOTYPE OF HUMAN COLON CANCER CELL?

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A human colon carcinoma cell line, HT29, was found, by Scatchard analysis, to express cell surface urokinase (u-PA) binding sites ($1x10^5$ / cell, Kd 1,5 nM).In contrast, no u-PA could be detected in the supernatant of the cells, as shown by zymographic analysis and radioiodinated fibrin plate assay. Furthermore, when HT29 cells were incubated with human plasminogen, they were unable to generate active plasmin. These results indicate the absence of surface bound u-PA on HT29. Preincubation of cells with purified two chain high molecular weigth u-PA followed by incubation with plasminogen demonstrated significant plasmin generation, as measured on radiolabeled fibrin plates. As a control, low molecular weight u-PA incubated with HT29 was unable to generate active plasmin in this assay. These results demonstrate that tumor cells expressing binding sites for u-PA are capable of initiating a PA plasmin-mediated proteolytic cascade.associated with the cell surface. This model cell line should allow us to further investigate the role of the u-PA receptor in tumor invasion and metastasis in vitro as well as in vivo.
Supported by the Commission of the European Communities (LR) and by grant from the Swiss Science Foundation.

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THE INFLAMMATORY RESPONSE OF THE LIVER IS SEVERELY REDUCED DURING AGING.

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We are interested in the inflammatory response of the liver, or acute phase, in aging animals. To investigate whether or not this response is compromised during aging, we have isolated several cDNA clones containing sequences induced during acute inflammation. Northern blots have shown that adult rats (10 month old) respond to an inflammatory stimulus by strongly increasing transcription of these acute phase genes. In contrast, this response is severely reduced in old animals (24 month old). Indeed, most of the genes tested are less induced in old than in young animals. In some cases (haptoglobin and an as yet unidentified acute phase gene), turpentine injection simply fails to induce a response in aged animals. To understand the molecular basis of this phenomenon, we have optimized culture conditions for primary cells from both young and old animals, and have been able to obtain cell cultures that express high levels of albumin, while maintaining a low basal level of expression of acute phase genes, such as MAP and AGP. We are now testing the possibility of inducing the acute phase response in these cultured systems, by adding IL-6 into the culture medium.

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IDENTIFICATION OF NOVEL TRANSCYTOSING MOLECULES IN THE CANINE KIDNEY EPITHELIAL CELL LINE MDCK

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We have established a biochemical assay to detect apical glycoproteins move by transcytosis to the basolateral membrane. A mutant cell line derived from MDCK cells, MDCKII-RCA^r, is labeled with [^{3}H]galactose by apical exogalactosylation at 4°C, and chased at 37°C to allow internalization of labeled glycoproteins. Delivery of [^{3}H]-labeled glycoproteins to the basolateral membrane was detected by selective biotinylation. Detergent-solubilized biotinylated glycoproteins were adsorbed onto streptavidin-agarose. Seven apical glycoproteins were transported selectively to the basolateral membrane as identified by 2D-IEF/SDS-PAGE. Transcytosis was time-dependent with half-times for arrival at the basolateral membrane of ~1-2 h. Incubation at 20°C completely blocked transcytosis. Depolymerization of microtubules delayed basolateral delivery. A monoclonal antibody was raised that immunoprecipitates an 80-kD and an 47-kD transcytosing glycoportein.

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The Human Asialoglycoprotein Receptor Specifically Binds a Plasma Membrane Adaptin

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The first step in receptor mediated endocytosis is the clustering of specific receptors with or without bound ligands in clathrin coated pits/coated vesicles. Mutations in the cytoplasmic tail of the LDL receptor prevent clustering in coated pits and endocytosis. Recently, it has been demonstrated that the tails of receptors interact with a class of clathrin coated vesicle proteins called adaptins. We have developed an *in vitro* assay to examine the interactions between the cytoplasmic tail of the ASGP receptor and adaptor proteins. The tail of the receptor specifically interacts with the plasma membrane-specific adaptins but not the Golgi-specific adaptins. This assay will be a useful tool to determine the structural requirements for this interaction.

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SEMLIKI FOREST VIRUS INDUCED CELL-CELL FUSION AT NEUTRAL EXTRACELLULAR pH

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We have previously described a system comprised of Aedes albopictus cells (clone C6/36) infected with Semliki Forest virus (SFV) which can be stimulated to undergo cell-cell fusion at 16 hours post infection at mildly acidic pH (<6.2). This polykaryon formation is known as a fusion from within (FFWI). The initial step is triggered by a conformational change of a surface protein, most probably a virus-coded protein. Concomitantly with the conformational change an influx of protons was detected. Sofar it is unclear whether this observed acidification might be important for the fusion process. Using NH_4Cl pulses and covalent modification of surface proteins we demonstrated that SFV-induced FFWI, which normally occurs only at low extracellular pH, can also be triggered by a transient acidification of the cytoplasm of infected cells at an extracellular pH of 7.4. These results imply a revision of the current view of SFV induced cell-cell fusion.

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ADHESION DURING NORMAL DEVELOPMENT AND MALIGNANCY Tucker, G.C., Duband, J.L., Dufour, S., Boyer, B., Valles, A.M., Gavrilovic, J., Jouanneau, J., Moens, G. and Thiery, J.P., CNRS and Ecole Normale Supérieure, Laboratoire de Physiopathologie du Développement, 46 Rue d'Ulm, 75230 PARIS Cedex 05, FRANCE

Cell locomotion mediated by extracellular matrix components of basement membranes and connective tissues has been demonstrated to play a crucial part in normal development, and has also been invoked as a prerequisite to invasion during metastatic spread. Two model systems will be briefly introduced to illustrate the role of adhesive molecules during embryogenesis and metastasis. In the first model, we analysed the individualization of embryonic neural crest cells and the mechanisms leading to emigration from the neural tube and translocation into well-defined pathways of migration. Evidence has been obtained to acknowledge the role of at least one component in neural crest cell migration, but even for fibronectins, there are many unanswered questions. The second model deals with the first steps of the metastatic cascade. Two distinct ways of promoting dispersion of bladder carcinoma will be discussed : one involves the extracellular matrix - through collagenous interactions - and the second, which can act in synergy with the first one, ascribes a new role as scatter factor to a family of growth factors. It is hoped that such models will help to understand the complex processes of regulation and modulation of cell adhesion in developmental and malignant states.

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THE STRUCTURE OF THE DNA-BINDING DOMAIN OF THE 434 REPRESSOR IN AQUEOUS SOLUTION DETERMINED BY NMR.

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The three-dimensional structure of the N-terminal domain of the 434 repressor (residues 1-69) has been determined by NMR spectroscopy in solution. It includes 5 helices, which are tightly packed together, and a flexible C-terminal segment. The helices 2 and 3 form a helix-turn-helix motif, which has been shown to be responsible for the sequence-specific DNA recognition. The structure is overall very similar to the one observed in single crystals (Mondragon et al., 1989, J. Mol. Biol. 205, 189-200). In the structure determination we made use of several novel NMR experiments which were developped with different isotope-labeled preparations of the 434 repressor (Neri et al. (1989) Biochemistry 28, 7510-7516; Wider et al. (1989) J. Magn. Reson. in press; Neri et al., J. Am. Chem. Soc., submitted).

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STRUCTURE AND ORGANISATION OF EF-1α GENES IN SOYBEAN

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Using a EF-1α (tef) gene probe from Euglena gracilis (P.E. Montandon and E. Stutz, NAR, 1990) we screened a cDNA library from soybean and identified and sequenced the coding part, which shows very high sequence identity with other eukaryotic EF-1α genes. Homologous DNA probes were used to search for tef genes in a genomic library. Two tef genes per haploid genome exist, both having a small intron in the coding part. The two genes are about 3 kb apart having the same orientation. Tef gene expression in function of light induction (etiolated → green plants) was monitored. Contrary to observations made with Euglena, tef gene(s) expression is susceptible to light induction at certain stages of development. We presently study mechanisms involved in induced tef gene expression.

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RAPID ISOLATION AND N-TERMINAL SEQUENCE DETERMINATION OF SPECIFIC DNA-BINDING PROTEINS AT FEMTOMOLE QUANTITIES

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One important class of sequence-specific DNA-binding proteins, the transcription regulators, are usually present only in small amounts. We have developed a method which allows to purify specific DNA-binding proteins or their DNA-binding domains from small amounts of cells and to determine their amino terminal sequence at femtomolar quantities. Nuclear proteins are UV-crosslinked to oligonucleotides containing the binding site and a dA tail and the complexes are purified on a dT-cellulose column. After separation by SDS-PAGE, individual protein-DNA complexes are blotted to glass fiber filters and submitted to microsequencing,using a fluorescence detection method which has the following advantages: a) high sensitivity, b) possibility to sequence DNA-protein complexes. Using this novel approach, we have isolated and directly sequenced the DNA-binding domain (=homeodomain) of mouse OTF-1. We are now characterizing octamer binding proteins from other species and different cell types.

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Isolation of a gene encoding a potential nuclear pore protein of yeast *Saccharomyces cerevisiae*

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We have isolated a potential nuclear pore protein of yeast by the following criteria: 1. It is recognized by a monoclonal antibody specific for rat nuclear pore proteins (Snow et al., JCB 104,1143). 2.It binds wheat germ agglutinin and tRNA. Partial amino acid sequence was obtained by microsequencing of tryptic peptides. The sequence of the peptides displayed many serine and threonine residues. Corresponding synthetic oligonucleotides were used to screen a genomic library . One clone, hybridizing to three independent oligonucleotides was sequenced. We found an open reading frame capable of encoding a 42 kDa protein very rich in serine and threonine. Essentially all peptide sequences were detected in the gene. Functional characterization of the 42 kDa gene is in progress.

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FUNCTIONAL ANALYSIS AND COMPARISON OF SIX YEAST TRANSCRIPTIONAL TERMINATORS

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The formation of 3'ends of yeast mRNA seems to be different from higher eukaryotic cells, since [1] most yeast genes lack the highly conserved mRNA processing signal AAUAAA and [2] no transcription that goes downstream the polyadenylation site can be detected. In our studies we investigated putative transcriptional terminators from six yeast genes. We constructed a termination test plasmid carrying a fusion gene with part of the actin gene (promoter,exon1,intron and part of exon2) plus URA3 as reporter gene. The six terminator fragments, all spanning a 200-300 bp DNA sequence from the end of the coding region to at least 20 bp downstream the polyadenylation site, were cloned into the intron of our construct. The effects of the terminators were analysed by Northern hybridisation and S1 mapping. We found different classes of termination sequences: [1] the GCN4 and PHO5 sequences act as strong terminators in a strictly orientation dependent manner, [2] the ADH1 fragment was able to shut down transcription completely in either orientation and [3] the TRP1, TRP4 and ARO4 terminators had a significantly reduced efficiency and functioned in either orientation when inserted in our test plasmid. Therefore our results support the assumption that 3'ends of mRNA in yeast might be formed by more than one mechanism.

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A LABIAL-LIKE HOMEOBOX FROM THE NEMATODE *CAENORHABDITIS ELEGANS*

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A new homeobox-containing gene (ceh-13) of *C. elegans* was isolated by screening an egg cDNA library at low stringency with the homeobox ceh-11. The homeodomain encoded by ceh-13 was found to be most similar to homeodomains belonging to the labial class, namely to the murine hox-1.6 with 43 amino acids identical out of 60, and to the *Drosophila* homeodomain labial with 41 residues identical out of 60. Furthermore, the similarity between ceh-13 and labial extends somewhat downstream of the homeodomain, the amino acids 61, 62, 65 and 66 being identical. The presence of a labial-like homeobox as well as Antp-like homeoboxes (AHB-1 and mab-5) in a nematode suggests that at the time when the nematode lineage diverged from the myriapod-insect and the vertebrate lineages, the gene duplication which gave rise to the Antp and the labial families of homeoboxes had already taken place.The corresponding genomic clone was isolated and found to map very close to a second homeobox, Hom-2/3. This finding suggests that in *C. elegans* some homeobox-containing genes are organised in "mini-clusters" comparable to the larger clusters known to exist in vertebrates, insects and annelids.

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TWO HIGHLY ABUNDANT SATELLITES IN THE GENOME OF *PANAGRELLUS REDIVIVUS*

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Several short *Eco*RI restriction fragments of the nematode *P. redivivus* have been cloned and analysed. Two major clone forms, 162 bp and 173 bp in length, were shown not to crosshybridize but to light up as typical satellite patterns on genomic Southern blots. Quantification of these satellites amounts to approximately 7% of the total genomic DNA for the 162 bp and to 10% for the 172 bp form. These figures are astonishingly high considering the relatively low C-value of *Panagrellus*, but the ratio of satellite to non-satellite DNA corresponds roughly to that found in the germ line of the chromatin eliminating nematod *A. lumbricoides*. No difference in satellite DNA contents could be detected between early larval DNA and adult DNA, far more enriched with germ line cells than the former. This finding might indicate that *Panagrellus* does not eliminate significant quantities of genetic material from somatic nuclei. The satellite sequences were also used to test genomic DNA for methylation by means of the restriction enzymes *Sau*3AI and *Mbo*I or *Msp*I and *Hpa*II. Again, the satellite probes failed to detect any difference between isoschizomer enzyme tracks, and, apparently, methylation does not seem to be a characteristic feature in the *Panagrellus* genome.

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PAT, TRANSPOSABLE ELEMENTS WITH AN UNUSUAL STRUCTURE

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PAT, a transposable element of *Panagrellus redivivus*, was identified after its insertion and thus the creation of a spontaneous mutation in the *unc* 22 gene of the nematode. Copy numbers per haploid genome range from 10 to 50, depending on *Panagrellus* strains, and the distribution of PAT elements is rather scattered. The predominant and presumably autonomous form is 5.8 kb long, but several internally deleted elements are also detected in the genomes. Preliminary single stranded M13 clone hybridization data show that PAT elements contain directly repeated sequences (DR's) at either side. Further analysis, however, revealed that they are not arranged as in typical retroviruses. Rather, one integral DR is found inside while another, split DR, is found one half at each end of the element. Configuration considering half DR's (A and B) is alternate (A....BA..B) in the great majority if not all the elements. Therefore, this does not reflect an event of separate transposable elements having integrated into or next to one another. Moreover, DR and half DR sequences seem always to be associated with internal, single copy element sequences, in contrast to the situation found for TOC1, Ty and other transposable elements.

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THE COMPLETE NUCLEOTIDE SEQUENCE OF THE RETROVIRUS-RELATED ELEMENT TAS PRESENT IN THE GENOME OF *A. LUMBRICOIDES*

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The proretrovirus-like element TAS is present in about 50 copies in the haploid genome of the nematode *A. lumbricoides* . One complete copy was sequenced. The putative *gag* region near the 5' LTR contains a 1962 bp long open reading frame which codes for a RNA binding domain. Near the center of the element we found on the +2 frame (relative to the *gag* frame) a region which shares some characteristics with known *pol* genes. However, this part of the sequence is interrupted by three stop codons. One of the putative polypeptides contains the amino acid sequence YVDN, a slightly changed version of the box YXDD which is present in almost all known reverse transcriptases. We conclude that stop codons as well as the change in the sequence of the YXDD box are the result of point mutations and that the sequenced element may represent a nonfunctional copy of TAS. Additionally, this YVDN box is carboxyterminally flanked by a conserved RNAse H and a zinc finger motif. The latter characterizes a possible endonuclease domain. A further 2061 bp spanning ORF near the 3' LTR of TAS corresponds to an *env* region. TAS represents obviously a retrovirus-related sequence with a similar gene arrangement as, e.g., that of the mobile element 17.6 in the *Drosophila* genome and that found in some genomes of retroviruses.

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A GENE CONTAINED WITHIN THE ELIMINATED CHROMATIN OF *ASCARIS LUMBRICOIDES*

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The segregation of the germ line and somatic cell lineages in the early embryonic development of *A. lumbricoides* is paralleled by the phenomenon of chromatin elimination.The eliminated material contains mostly highly repetitive sequences, but also some middle repetitive and single copy DNA. Recently,we succeeded in isolating a cDNA clone, *EGAL 1* (eliminated gene of *A. lumbricoides* 1), containing eliminated, single copy sequences. *EGAL 1* has a length of 540 bp and contains an ORF coding for a 148 amino acids long protein. This protein shows a significant homology to the ribosomal protein S16 of *S. cerevisiae*, with respect to size, amino acid sequence and high content in Arg and Lys. Northern blot analysis demonstrates that the RNA transcripts of *EGAL 1* are present in oocytes as well as in 4-cell embryos up to the elimination stages, but not in later larval stages. The cDNA of *EGAL-1* hybridizes to three single copy bands within the germ line genome of *P. equorum*, another chromatin eliminating nematode. Interestingly, like in *Ascaris*, these DNA sequences are missing from the somatic cells. Obviously, not only the sequence of *EGAL-1* is conserved between the two nematodes, but also its behaviour during the chromatin elimination process.

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PROTEIN DISTRIBUTION AND PROMOTER ANALYSIS OF THE *DROSOPHILA GOOSEBERRY* LOCUS

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One of the first developmentally important events during *Drosophila* embryogenesis is the division of the embryo into metameric units. This process has been shown to depend on zygotically expressed segmentation genes. In the absence of any of these genes, embryos die and exhibit characteristic defects in their cuticular pattern. Segmentation genes have been grouped, according to their cuticular mutant phenotypes, into three classes, the gap, pair-rule, and segment-polarity genes. The *gooseberry* (*gsb*) locus belongs to the third class and encodes two transcripts which are expressed in overlapping sets of cells in the posterior half of each segment. In the absence of *paired*, both *gsb* genes fail to be induced in every other segment, suggesting a role for *prd* as an activator of the *gsb* genes. Since only deletions of the *gsb* locus that inactivate both *gsb* genes are available and since the upstream regions of the two *gsb* genes abut or even overlap, several models of regulatory interactions are consistent with the genetic evidence. We will discuss these models in the light of precise immunolocalizations of both *gsb* proteins as well as of results obtained from a recently initiated promoter analysis of the two *gsb* genes.

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REGULATION OF THE *DROSOPHILA PAIRED* GENE BY PAIR-RULE GENES

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The patterns of transcripts of the *Drosophila* pair-rule gene *paired* (*prd*) have been analyzed in all single and in a number of double pair-rule mutants at syncytial and cellular blastoderm. From their comparison with the *prd* patterns observed in wild-type embryos, a complete regulatory scheme of how the pair-rule genes control *prd* activity has been derived. The scheme illustrates several salient features of *prd* regulation by the other pair-rule genes. It explains how the transient early *prd* pattern exhibiting a double-segment periodicity at syncytial blastoderm is altered, by the combinatorial action of the remaining pair-rule genes, into a pattern with a single-segment repeat . Secondary pair-rule genes like *odd-paired* and *odd-skipped* act directly on *prd* while the primary pair-rule genes *hairy, runt, and even-skipped exert their effect on prd* indirectly via secondary pair-rule genes. Thus, the *prd* gene is located at the bottom of a hierarchical regulatory network of pair-rule genes and mediates the transition of positional information from the pair-rule to the segment-polarity genes.

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ANALYSIS OF UPSTREAM ELEMENTS CONTROLLING *E(spl)* GENE EXPRESSION

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In *Drosophila*, the adoption of neural versus epidermal fate is dependent on the action of the so called neurogenic loci. Molecular analysis of one of these loci, *Enhancer of split [E(spl)]*, revealed a homology of *E(spl)* gene products (m9/m10) to mammalian ß- transducin. Focusing on the m9/m10 promoter region we have determined the initiation site of transcription. In order to identify control elements the upstream region will be fused to a reporter gene (ß- galactosidase) and introduced into flies via P-element mediated transformation. ß- gal activity in transgenic embryos reflects the regulatory capacity of the fragments used in the gene fusions and can readily be monitored. Bal31 or ExoIII deletions in the upstream region will allow us to identify the minimal DNA sequences necessary to mimic normal *E(spl)* expression. These sequences will be subsequently used in a footprint analysis to determine binding sites of trans- regulatory factors.

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TRANSCRIPTION FACTOR Oct-2A CONTAINS REDUNDANT ACTIVATING DOMAINS AND WORKS FROM A PROMOTER BUT NOT FROM A REMOTE ENHANCER POSITION IN NON-LYMPHOID (HeLa) CELLS

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The "octamer" motif ATTTGCAT is found in all immunoglobulin (Ig) promoters and in the Ig heavy chain (IgH) enhancer. B cell-specific transcription is critically dependent on the presence of the octamer motif. Also, we already showed that lymphoid-specific octamer containing promoters can be activated in non-lymphoid (HeLa) cells by the cloned cell type-specific "octamer" binding transcription factor Oct-2A.

Deletion analysis of the cDNA coding for Oct-2A reveals a glutamine-rich region in the N-terminus which is important for transcriptional activation as shown with two different promoters. The promoters respond differently to the N-terminal deletions while another deletion mutant, consisting essentially of the complete POU-domain, is able to weakly transactivate only one of them. Surprisingly in HeLa cells, the cloned Oct-2A protein does not transactivate from an artificial lymphoid specific and octamer containing enhancer at a remote position. This observation is also of interest because (i) the octamer sequence has been proposed to be involved in transcriptional extinction and (ii) the activity of Pit-1, also a POU gene, appears to be specifically downregulated in non-permissive cells.

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FUNCTIONAL CHARACTERIZATION OF THE OCT-1 AND OCT-2 PROTEIN

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Immunoglobulin (Ig) genes are expressed in B lymphocytes, but not in non-B cells. An important component of this B-cell specificity is the conserved octamer sequence ATGCAAAT, present in the promoter and the enhancer. The octamer motif is recognized by the two B-cell specific factors Oct-2A and Oct-2B and the ubiquitous Oct-1 protein. In vitro, the three Oct factors show no difference in binding to either the octamer sequence from Ig genes or the same motif in non-lymphoid specific promoters such as the histone H2B promoter. Why then does the Oct-1 protein not activate a B-cell specific promoter in non-B cells? We are addressing this question by performing experiments with the cDNAs for the Oct-2 protein (Müller et al., Nature 336, 544-551,1988) and the Oct-1 protein (kindly provided by W. Herr). Recently it was shown that a promoter consisting of an octamer element and a TATA box can be activated in non-B cells by coexpressing the Oct-2A cDNA. Surprisingly, preliminary results show that the same promoter can also be activated by cotransfection of the Oct-1 cDNA, though to a lesser extent. These results have left open the question if the regulation of Ig gene expression is achieved by quantitative or qualitative differences between the Oct-1 and Oct-2 proteins. For a more refined analysis we have initiated transfection experiments with Oct-1 deletion mutants and of domain swap experiments between Oct-1 and Oct-2.

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THE DNA BINDING DOMAIN OF OCTAMER TRANSCRIPTION FACTORS IS CONSERVED IN EVOLUTION

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Octamer transcription factors (OTF) are involved both in tissue-specific transcription of immunoglobulin genes, and in transcription of ubiquitously expressed genes and bind to the sequence ATGCAAAT(TA), which is conserved in many promoter and enhancer elements. With our new method, we could isolate several distinct OTFs from nuclear extracts of mouse and *Xenopus*. Digestion with different proteases showed a resistent DNA-binding domain, that had the same size as the DNA-binding motif "homeodomain". Indeed the partial aminoacid sequences of some of the isolated protein fragments are identical with the human POU-homeobox region and confirm the assumption that the homeodomain is a stable domain, conserved through evolution as a DNA-binding structure. With oligonucleotides derived from these partial protein sequences, we could isolate mouse cDNA clones that have very good homology to the HOMEO-POU region of human OTF-2. Outside the DNA-binding domain the homology is weaker. We claim that the DNA-binding domain of the protein was exposed to stronger evolutionary pressure than the other parts.

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DBP-2, A TRANSCRIPTIONAL ACTIVATOR INVOLVED IN LIVER-SPECIFIC GENE EXPRESSION

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The rat albumin promoter contains 6 binding sites (A to F) for DNA binding proteins, most of wich have been identified. Among these elements sites B and D are particulary important in conferring efficient liver-specific transcription to the albumin promoter. Element D binds multiple alternative factors including the proteins C/EBP (Landschulz et al. 1988. Gen. and Dev. 2, 786-800), DBP-1 (Mueller, C and Schibler, U. 1990. Cell, in press) and DBP-2. Most of the D-binding activity in liver nuclear extract can be attributed to DBP-2. Recently a cDNA encoding full-length DBP-2 (36 kD) has been cloned in our laboratory. This protein, when over-expressed in E. Coli, significantly enhances transcription by specifically binding to element D. DBP-2 mRNA (1.6 kb) accumulates in many somatic tissues but is absent from testis. In contrast, DBP-2 protein is considerably more concentrated in liver as compared to other tissues.

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CYTOSINE METHYLATION IN CTF AND Sp1 RECOGNITION SITES OF AN HSV tk PROMOTER : EFFECTS ON TRANSCRIPTION IN VIVO AND ON FACTOR BINDING IN VITRO

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We methylated specific cytosine residues within or immediately around the CTF and Sp1 binding sites of the Herpes simplex virus thymidine kinase promoter. The efficiency of transcription *in vivo* was reduced at least 50-fold compared with transcription from the unmethylated promoter. However, methylation within the CTF recognition site had no effect on the affinity of CTF for this site *in vitro*. Methylation of the Sp1 site resulted in only a small decrease in the affinity of this factor for its recognition site. In vivo studies showed that the same gene inserted in different vector DNAs was regulated differently by methylation in the promoter. These results show that cytosine methylation can inhibit transcription by a mechanism other than directly blocking the binding of transcription factors.

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Sp1 BINDING SITES UPSTREAM OF THE VITELLOGENIN GENE A1 PROMOTER AND TRANSCRIPTION INITIATION AT A SECONDARY SITE IN VITRO.

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Functional analysis of the 5'-end region of the *X. laevis* vitellogenin gene A1 revealed the presence of two initiation sites (i and io).In hepatocytes,transcription from the i site is 50-fold stronger than from the io site and is dependent, at both sites,on the estrogen responsive element.In contrast,in an estrogen-independent *in vitro* transcription system, using a Hela nuclear extract, the transcripts emanating from the io site were much more abundant. The presence of three sequence elements homologous to the Sp1 binding site immediately upstream of the io initiation site could account for this observation. We first demonstrated that Sp1 can interact with these sites in the A1 promoter.We then analysed the capability of the Sp1 protein present in various cell extracts to transactivate the expression of the A1 gene *in vitro*.

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MOLECULAR COOPERATION AND INTERFERENCE IN HORMONE-DEPENDENT TRANSCRIPTIONAL CONTROL

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We have studied the protein-protein and protein-DNA interactions occurring during transcriptional activation in model systems (*in vivo* and *in vitro*) involving the glucocorticoid receptor (GR) and its DNA target site (GRE). We demonstrate that GRE clusters can serve as very efficient and totally GR-dependent promoters or enhancers and that GR-mediated transcriptional control does not require a *bona fide* TATA box. Our results suggest also that direct interactions between remote enhancers and the target promoter may not be required. Furthermore, we have data suggesting that the architecture of the GRE cluster determines the efficiency of cooperation among the bound transactivators and that a rough correlation may exists between spatial organization of the GREs and the molecular size of the transactivator. Surprisingly, a minimal portion of the rat GR (aa. 407-556 including the zinc fingers) is sufficient for transcriptional activation over large distances. We also observed a strong interference of active GR with the function of the lymphocyte-specific octamer binding factor, a phenomenon which might be related to the immuno-spppressive properties of glucocorticoids. Systematic mutagenesis of the first zinc finger and surrounding regions has yielded some remarkable null-or semi-permissive mutants in which the target specificity seems to have been altered. Finally, a direct blotting technique has been developed, which allows for the visualization of strong protein-protein interactions involving the GR and other cellular components, like for instance the 90 kd heat shock protein.

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TISSUE-SPECIFIC TRANSCRIPTIONAL REGULATION OF MOUSE MAMMARY TUMOR VIRUS

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Epithelial cells of the mammary gland are not the only target for the tumorigenicity of Mouse Mammary Tumor Virus (MMTV), and several observations suggest that it might be involved in kidney and thymic cell transformation. The most obvious difference seen between MMTV–M (mammary origin) and MMTV–K (kidney origin) is located in the U_3 part of the LTR, just upstream of the glucocorticoid responsive region of the promoter. To test if the difference between the two viruses within the LTRs was controlling cell-specific gene expression, several approaches have been chosen: 1. Transfection of the proviruses and of constructions of both LTRs with a reporter gene (HSV TK) into kidney and mammary cell lines. 2. *In vitro* transcription of a template made of one or the other LTR using a reporter G-free cassette with various tissue and cell extracts. In parallel, DNA binding activities of these extracts have been tested using DNAseI footprints and band-shifts experiments. 3. Transfection, in different cell lines, of several constructions carrying different oligonucleotides in front on a heterologous promoter (HSV TK). And 4. Transgenic mice carrying the entire proviruses.

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REGULATORY INTERACTIONS IN THE STEROID CONTROL REGION OF MOUSE MAMMARY TUMOR VIRUS DNA.

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Transcription of MMTV DNA is stimulated by several steroid hormones. Using chimeric MMTV plasmids with mutations in the 200 bp upstream of the RNA start site, we showed differential contributions of regulatory elements in the response to progestins versus glucocorticoids. Functional interactions between elements were studied with spacing and sequence-duplication mutants. Binding of nuclear protein extracts in vitro showed the interaction with MMTV DNA of the glucocorticoid receptor, and of tissue-specific proteins in the 5' flanking region of the distal site and in the basal promoter region. Cells expressing the progesterone receptor showed significant differences in the in vitro interactions and are being analyzed by in vivo footprinting. We speculate that tissue specific factors may cooperate with the receptors to produce a cell-type dependent modulation of hormone action.

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A NUCLEAR FACTOR-I-LIKE ACTIVITY IS REQUIRED FOR THE ESTROGEN-REGULATED IN VITRO TRANSCRIPTION FROM THE XENOPUS VITELLOGENIN B1 PROMOTER

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Steroid-responsive elements are generally located at a distance upstream of the TATA box and often associated with binding sites of general transcription factors. In addition to the already well-documented estrogen responsive element (ERE; NT -334/-302), several CCAAT boxes have been detected within the Xenopus laevis vitellogenin B1 promoter. One of these binding sites is located within the basal promoter element (BPE) around position -100 and two others reside on both sides of the ERE. A factor similar to the human CTF/NF-I is present in Xenopus liver nuclear extracts and is absolutely required for the induction of the B1 gene. DNAase I footprinting experiments show that this factor as well as the purified human CTF/NF-I are able to bind to the three sites. Moreover, the ability of each NF-I-binding site to modulate the hormonal induction is independently analysed.

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mos-INDUCED DOWN-REGULATION OF GLUCOCORTICOID RECEPTOR FUNCTION IS MEDIATED BY Fos

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Expression from two glucocorticoid responsive promoters, MMTV LTR and metallothionein IIA, is only transient in cells expressing v-*mos* or *fos*. v-*mos* expression induces *fos* mRNA and functional Fos protein. 2-aminopurine which specifically inhibits *fos* expression abolishes the inhibition of glucocorticoid receptor dependent expression from the MMTV LTR promoter in v-*mos* expressing NIH 3T3 cells. Furthermore, *sof* expression inhibited the down-regulatory effect of *mos* on expression of a transiently transfected MMTV LTR-CAT in these cells. The formation of specific nuclear complexes between Fos and the glucocorticoid receptor is demonstrated. These findings suggest that Fos mediates the down-regulation of glucocorticoid receptor function in cells expressing the v-*mos* oncogene.

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IN VIVO AND IN VITRO HORMONAL REGULATION OF THE VITELLOGENIN B1 PROMOTER

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In oviparous vertebrates, vitellogenin, the precursor of the major yolk proteins, is synthesized in the liver of mature females under the control of estrogen. However, in *Xenopus* male animals, the silent vitellogenin locus can be activated de novo by administration of estrogen. In vitro analysis of the *X. laevis* vitellogenin B1 promoter in male liver nuclear extracts revealed three different regulatory elements located within 340 bp which drive tissue-specific transcription. Two of these elements, the basal promoter element (BPE) and the negative regulatory element (NRE), were found to be involved in the tissue-specific expression of the gene, while the third one is the estrogen-responsive element (ERE).With the aim to correlate these in vitro data with in vivo results, we established the first transfection system using *X. laevis* hepatocytes primary cultures. The in vivo mapping of the promoter elements is in agreement with the in vitro data, with additional positive regulation of regions located further upstream.

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XENOPUS LAEVIS ESTROGEN RECEPTOR GENERATED FROM A VACCINIA RECOMBINANT VIRUS: BINDING AND FUNCTIONAL ANALYSIS.

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The X. laevis estrogen receptor (ER) is a transcription activator required for the hormonal induction of the vitellogenin genes. The full-length cDNA encoding the *X.laevis* ER has been recombined into a vaccinia virus vector and the activity of the recovered protein was assayed by band-shift and in vitro transcription. We first determined that the protein is capable to interact with the ERE of the vitellogenin A2 or B1 promoter. Second, we demonstrated that the ER present in crude extracts prepared from infected cells can potentiate transcription. Finally, the transactivatory capacity of heparin-sepharose fractions was analyzed by complementation of Xenopus male liver nuclear extracts that normally do not transcribe the vitellogenin promoter.

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FUNCTIONAL ANALYSIS OF XENOPUS ESTROGEN RECEPTOR EXPRESSED IN VACCINIA VIRUS

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Steroid hormone regulated gene expression is transcriptionally controlled by steroid hormone receptors. We study the estrogen dependent transcription of vitellogenin genes in *X. laevis* and analyse the following questions:
- How does estrogen bind and activate the receptor?
- How does this activated complex interact with DNA and initiate transcription?
- How do antagonists inhibit these interactions?

A recombinant vaccinia virus which expresses the Xenopus estrogen receptor was first constructed and used to infect HeLa cells. We analysed the expression of [^{35}S]-methionine labelled receptor by SDS-PAGE and immunoprecipitations. The receptor/DNA interactions were analysed with whole cell extracts or with partially purified receptors by bandshift and electron microscopy in the presence or absence of estrogen or antagonists. The stability of the receptor/DNA complex was examined by varying salt concentrations and the amounts of nonspecific DNA.

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COOPERATIVE BINDING OF ESTROGEN RECEPTOR TO IMPERFECT ESTROGEN-RESPONSIVE DNA ELEMENTS CORRELATES WITH THEIR SYNERGISTIC HORMONE-DEPENDENT ENHANCER ACTIVITY.

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The Xenopus vitellogenin gene (vit) B1 estrogen-responsive unit (ERU) is an enhancer formed by two adjacent 13 base pairs (bp) imperfect palindromic estrogen-responsive elements(ERE-1 and ERE-2), having one or two bp substitutions when compared to the perfect palindromic consensus ERE (GGTCANNNT-GACC). These degenerated EREs, on their own, have a low or no regulatory capacity at all, but in vivo act together synergistically to confer high receptor- and hormone-dependent transcription activation to heterologous promoters. Using in vitro protein-DNA interaction techniques, we show that estrogen receptor dimers bind cooperatively to the imperfect EREs of the vit B1 ERU. Binding of a first receptor dimer to the more conserved ERE-2 increased 4- to 8-fold the binding affinity of the receptor to the adjacent less conserved ERE-1. Thus,the observed synergistic transcription activation may be the result of cooperative receptor binding to these EREs.

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THE IRF-MEDIATED PATHWAY OF VIRUS INDUCIBILITY

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Sequences occurring within (GAAANN)$_4$ are frequent in the virus responsive sequences of the IFN-α1 and-β promoters (termed VREα1 and VREβ, respectively. (AAGTGA)$_4$ or (GAAAGT)$_4$ mediate virus inducibility when placed upstream of a minimal promoter and bind a factor, IRF-1 (Myamoto et al., Cell **54**, 903), which also binds to VREβ but only weakly to VREα1. We show that the sequence within such multimeric repeats interacting with IRF-1 is GAAAGTGAAAG, which closely resembles the PRDI sequence characterized as a virus responsive element in VREβ (Fan and Maniatis, EMBO J. **8**, 101). IRF-1 is present at very low levels if at all in uninduced L cells, and is strongly induced by virus infection or IFN treatment, which led to the suggestion that IFN induction is a consequence of IRF-1 synthesis (Harada et al., Cell **58**, 729). However, cycloheximide does not inhibit viral induction mediated by (GAAAGT)$_4$ or VREβ under conditions where IRF-1 synthesis is inhibited. Either "masked" IRF-1 is released and/or activated, or there is an alternative induction pathway. Although (GAAAGT)$_4$-mediated transcription is stimulated over 20 fold by cotransfection with an IRF-1 expression plasmid, VREβ is stimulated only 2.7 fold and VREα1 not at all. Activation of the IFN-β promoter may require simultaneous binding of IRF-1 to PRDI and NF-κB to the NF-κB binding site PRDII, and perhaps release of a repressor (Lenardo et al., Cell **57**, 287). Our results suggest that virus induction mediated by VREα1 involves neither IRF-1 nor NF-κB, and we have identified a different virus responsive element within VREα1, the "TG" sequence, which is absent in VREβ (Maguire et al., this volume).

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DIFFERENT PATHWAYS MEDIATE VIRUS INDUCIBILITY OF THE HUMAN IFN-α AND IFN-β GENES

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Virus inducibility of the IFN-α1 and IFN-β genes is mediated by different response elements. Activation of the IFN-β promoter apparently requires release of a repressor(s) and binding of two factors, IRF-1 and NF-κB (Myamoto et al., Cell **54**, 903; Lenardo et al., Cell **57**, 287). The virus responsive element of IFN-α1 (VREα1) binds IRF-1 very weakly and NF-κB not at all. We propose that inducibility of VREα1 is mediated at least in part by a different factor which binds the "TG" sequence, GAAATGGAAA. This is supported by the finding that an A->C mutation (underlined) in the VREα1 TG sequence reduces inducibility more than 7 fold. Also, oligos with the TG sequence mediate inducibility by virus but not IRF-1, in contrast to the IRF-binding element derived from VREβ which is both virus and IRF-1 inducible. Two major proteins binding to VREα1 have been identified by gel retardation assay, the ubiquitous octamer factor OTF-1 and the novel "TG protein". Since dimeric octamer binding site is not virus inducible, it is unlikely that octamer factors mediate virus inducibility and a more likely candidate is TG protein. Competition experiments show that binding of TG protein is not competed by IRF-1 or NF-κB binding oligonucleotides, or by VREβ. Methylation interference using partially purified TG protein confirms that it binds specifically to the TG sequence. Thus the TG sequence represents a virus inducible element distinct from the NF-κB and IRF-1 sites of VREβ.

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ERYTHROPOIETIN REGULATION: A 50K PROTEIN WITH AN OXYGEN-DEPENDENT DNA-BINDING PROPERTY

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HepG2 cells can be used to study the O_2-dependent regulation of erythropoietin (EPO). We analysed nuclear proteins of normoxic and hypoxic HepG2 cells for their specific binding to a DNA fragment in the upstream region of the EPO gene. A 50k protein with a DNA-binding activity that could be modulated by metal ions was detected. $ZnCl_2$ promoted whereas $CoCl_2$ suppressed protein-DNA interaction, irrespective of the O_2-pressure the cells had been exposed to. In presence of $FeCl_3$ the DNA-fragment was bound to the protein isolated from normoxic, but not to that from hypoxic cells. This provides evidence that the 50k protein is an iron protein with a O_2-modulatable DNA-binding activity acting as a repressor on EPO gene transcription under normal conditions. This is supported by the fact that $ZnCl_2$ and $CoCl_2$ affected EPO synthesis in HepG2 cells as expected from the data from the DNA-binding analysis.

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EPITHELIAL CELL-SPECIFIC MEMBER OF THE JUN-FOS AP1 FAMILY OF FACTORS REQUIRED FOR TRANSCRIPTION FROM A HUMAN PAPILLOMA VIRUS TYPE 18 PROMOTER.

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Papillomaviruses are tissue-specific and replicate in differentiating keratinocytes. We are interested in the question of tissue specificity at the level of transcription. We used nuclear extracts from human foreskin keratinocyte and fibroblast cells and from HeLa cells to look for factors binding to the E6 promoter of HPV-18 DNA by footprint and gel mobility shift experiments. We found a factor present in HeLa and keratinocyte extracts but not in fibroblast extracts, which binds about 160bp upstream from the start of E6. The binding site includes the sequence, TGACTAAG, which resembles the consensus binding site for the Jun-Fos AP1 family of proteins. Synthetic oligonucleotides containing this binding site specifically compete the factor binding to HPV-18 DNA. They also inhibit transcription of E6 in extracts of HeLa cells in vitro. Thus the presence of this epithelial cell-specific factor seems to be important for HPV-18 transcription.

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COOPERATION OF V-HA-RAS AND GAG-V-MYC ONCOGENES IN ABROGATING THE IL-3 REQUIREMENT OF PB-3C MASTOCYTES

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The expression of oncogenes in hematopoietic cells can result in the abrogation of lymphokine requirement and tumor formation. We have previously shown, that Ha-ras oncogenes can shift the IL-3 requirement of an interleukin-3 dependent mast cell line (PB-3c) to lower concentrations and at very high p21 levels even to independence. The ras oncogene family has been demonstrated to cooperate with various nuclear oncogenes.
This work now presents evidence that in the PB-3c mast cells, v-Ha-ras is able to cooperate with the gag-v-myc oncogene leading to complete factor independence. Cells expressing either oncogene alone were still dependent on IL-3. Mass cultures and clones, derived from MMTV gag-v-myc transfections, superinfected with a v-Ha-ras retroviral vector became growth factor independent. Supernatants of 3 out of 7 IL-3 independent lines contained a mitogenic activity when tested on parental PB-3c cells. Using PCR technology, we are presently analyzing the nature of the mitogenic factor and the contribution of ras, myc or both oncogenes in its induction.

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IDENTIFICATION AND CHARACTERIZATION OF TWO C-MYC GENES FROM XENOPUS LAEVIS

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The c-myc proto-oncogene is well conserved in vertebrate evolution. The sequence of a partial cDNA clone of the 2.7 kb X.laevis c-myc mRNA has been previously reported by others. Two homologous c-myc genes named myc 1 and myc 2 have been isolated. Sequence comparisons revealed that myc 2 is encoding the previously isolated cDNA. The high degree of conservation between the two genomic clones myc 1 and myc 2 in the coding area confirms that X.laevis c-myc gene exists as a duplicated set. We have isolated and identified the two putative myc promoter regions and determined their sequences. Comparison of these X.laevis sequences with those of mouse or human reveals common features. Among them, the sequence of a negative regulatory element that affects the expression from both P_1 and P_2 human promoters. Construction of chimeric genes where the CAT coding sequence is under the control of the myc promoters and putative regulatory elements derived from the 5' upstream region the 1^{st} exon and the 1^{st} intron will now allow us to clarify the mechanisms controlling myc transcription in Xenopus oocytes and embryos.

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ONCOPROTEIN-MEDIATED INDUCTION OF GENE EXPRESSION

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Growth promoting agents which interact with target cells at the periphery trigger a cascade of events which ultimately lead to the transfer of signals into the nucleus. These signals cause the altered expression of a set of genes whose products are involved in DNA synthesis and mitosis. Oncogene products interfere with the normal flow of the growth promoting information. We hope to contribute to the elucidation of the signal transduction pathways onto which oncoproteins excert their action by studying the molecular mechanisms of oncoprotein-mediated gene expression. Two genes have been identified in mouse fibroblasts (T1, α B crystallin) whose expression is induced by the Ha-ras (EJ) and the v-mos oncogene products. The T1 gene encodes a 39 kDa protein which is related to the products of the immunoglobulin supergene family. Its sequence is most closely related to the one of carcinoembryonic antigen. In vitro transcription-translation experiments revealed membrane association or secretion as well as heavy glycosylation of the T1 gene product. The α B crystallin gene, which is most abundantly expressed in the eye lens but whose expression has also been detected in other organs is induced by the two studied oncogenes in mouse fibroblasts. Transcription was shown to initiate at the same site as in eye lenses. Promotor regions of the two genes (T1, α B crystallin) are available and we are currently studying the oncogene-mediated induction mechanism.

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IN VITRO MODULATION OF N-MYC AND MHC CLASS I and II GENE EXPRESSION BY HUMAN NEUROBLASTOMA CELLS

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Neuroblastoma (NB) cell lines and tumors are characterized by poor HLA class I antigenic expression. The majority of NB cell lines and a high percentage of disseminated tumors display amplification of the nuclear proto-oncogene N-myc. An inverse correlation between HLA class I antigenic expression and N-myc amplification and overexpression has been recently described in NB. We show here that N-myc and HLA RNA steady-state levels can be modulated independently and variably by cytokines, inducers of differentiation and growth factors. The effect of these substances on antigenic expression and cells growth has been likewise measured.

While both cytokines decrease the growth of NB cells and induce the expression of MHC antigens, only alpha-TNF is able to influence the levels of N-myc transcripts. In contrast differentiating agents usually cause a decrease of N-myc RNA levels without significant influence on MHC gene expression. Growth factors variably influence the growth of NB cells without significant antigenic changes after exposure to NGF or EGF. In contrast a stimulation in the expression of N-myc is obtained after exposure of NB cells to EGF.

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TRANSCRIPTION AND CAMP REGULATION OF THE Dd *ras* GENE.

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The Dd *ras* gene encodes a protein highly homologous to mammalian proto-oncogenic *ras* proteins. Multiple transcripts of this gene accumulate during *Dictyostelium* development. We will show that the three transcripts which originate from Dd *ras* differ in their 5' ends. In single starved cells, Dd *ras* transcription can be induced by external addition of cAMP. Under these conditions, however, one major transcript over-accumulates. This regulation of Dd *ras* by cAMP seems to occur both at the transcriptional and post-transcriptional level, as shown by a comparison between the results of run on experiments and RNA hybridizations. We propose a model in which an RNA secondary strucure may play a key role. The model allows the re-interpretation of previous confusing results using Dd *ras* deletions.

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TRANSCRIPTIONAL STUDIES IN THE "ORI"-REGION AND IN VICINITY OF EUGLENA GRACILIS CHLOROPLAST DNA

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Euglena gracilis chloroplast DNA contains a single origin of DNA replication (Koller & Delius, 1982, EMBO J. 1:995-998) which maps in the vicinity of a polymorphic site, composed of multiple short direct repeats (Schlunegger and Stutz, 1984, C.G. 8:629-634). We sequenced a DNA segment of about 10 kb, flanking the ORI region. We retrieved a major ORF of 350 codons, a gene for tRNA-Leu (Monfort and Stutz, 1986, NAR 14:3971) and an extremely A+T rich region (84%) composed of two direct repeats (920 n) separated by 320 n. The region was probed for transcription activity. The tRNA-Leu gene is transcripted into a pre-tRNA of about 600 nucleotides. DNA from the ORF 350 region interacts with a stable transcript of close to 900 n and DNA probes from the repeats interacts with a transcript of 1050 n. The putative 41 kDa protein coded by ORF 350 has no significant similarity with any protein sequence stored in SwissPROT, 11. From these results we conclude, that large parts of non-coding DNA in the vicinity of the ORI-region are transcribed with no obvious function. Since this region contains multiple ARS consensus sequences we are about to probe for ARS-function.

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THE α AND β PROTEIN PHOSPHATASE 2A CATALYTIC SUBUNIT GENES: SIMILAR STRUCTURE BUT DIFFERENT PROMOTERS

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Protein phosphatase 2A consists of a catalytic subunit (C) associated with one or two different regulatory subunits. Molecular cloning revealed two C subunit mRNAs (α and β) that are expressed in different amounts depending on the tissue or cell line. To investigate the basis for this differential expression of the two isoforms we cloned the Cα and Cβ subunit genes from a human genomic library. They both span about 30 kb and consist out of 7 exons with the introns intervening at the same positions. This structural conservation suggests they evolved by gene duplication. The promoter regions of both genes showed typical features of housekeeping genes, such as a lack of TATA and CCAAT boxes and an increased G+C content. Analysis of the promoter strength revealed that the different α and β mRNA levels are partially due to differential transcription.

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STRUCTURE OF A HUMAN CALMODULIN GENE

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Calmodulin in humans is encoded by a multigene family consisting of at least three separate members specifying divergent mRNAs (Wawrzynczak, E.J. and Perham, R.N. (1984) Biochem. Int. 9, 1051-1057; SenGupta, B. et al. (1987) J. Biol. Chem. 262, 16663-16670; Fischer, R. et al. (1988) J. Biol. Chem. 263, 17055-17062). Here, we report the cloning and sequencing of the human calmodulin gene related to the human calmodulin cDNA ht6 (Fischer, R. et al. (1988) J. Biol. Chem. 263, 17055-17062). This gene, named CaMIII, lies on two overlapping genomic clones, λhGH7 and λhGO2, and spans about 10^4 base pairs. It consists of six exons and the intron-exon organization is identical to the one observed in rat and chicken calmodulin genes. All intron-exon boundaries behave according to the GT/AG rule. As in many genes encoding Ca^{2+}-binding proteins the initiation codon ATG is separated from the coding region by an intron. The start site of transcription has been determined by primer extension and ribonuclease protection experiments. DNA sequences in the promoter and 5'-untranslated region are very GC-rich and do not have typical CAAT or TATA boxes. In the 3'-untranslated region, two putative polyadenylation sites can give rise to the 2.3kb- and the 0.8kb-mRNAs detected in Northern blot experiments. To date, the gene described here represents the first human bona fide calmodulin gene for which the complete structure has been determined.

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ISOLATION AND CHARACTERISATION OF cDNA CLONES CODING FOR SOYBEAN THIOREDOXINS.

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Thioredoxins are ubiquitous small (MW~12 000) redox proteins with an active center disulfide/dithiol. They participate in a number of cellular reactions as electron donors or as regulatory proteins. Chloroplasts of photosynthetic cells contain two forms of thioredoxins (m and f), functioning as regulatory proteins and distinguished by their interaction with specific target enzymes.
Full lengh cDNA clones of thioredoxins from several bacterial and mammalian sources have been reported. From higher plants only the cDNA from spinach chloroplast thioredoxin f is known.
We are interested in studying the genomic sequences coding for thioredoxins in the leguminose Glycine max. A cDNA library constructed from the poli(A)+RNA of 6-day old soybean seedlings in lambda gt11 was screened by hybridization with the spinach cDNA sequence coding for thioredoxin f. Five positive clones resulted from the screening in 40% formamide. The phage DNAs were isolated and the insertions excised for cloning and sequencing.

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Gene expression during the senescence of *Arabidopsis thaliana*

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In many annual plants such as *Arabidopsis thaliana* foliar senescence is induced upon the beginning of fruit development. Such a correlation is meaningful because the degradative process associated with senescence represents a basis for the supply of nutrients to the developping seeds. Whereas the mitochondria of senescent mesophyll cells remain in a functional condition to ensure the energy supply for metabolic processes, the chloroplasts are extensively degraded as demonstrated by the yellowing of leaves. All senescence specific changes in mesophyll cells are under strict genetic control and dependent on cytoplasmatic protein synthesis. Specific genes appear to be induced in senescent leaves. In our work we are investigating the induction of isocitrate lyase and malate synthase in the senescent rosette leaves of *A. thaliana*. These enzymes may be involved in the transformation of acyl residues produced during the degradation of thylakoid membranes into transportable sugars.

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NUCLEAR TRANSFORMATION OF THE GREEN ALGA CHLAMYDOMONAS REINHARDTII

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The gene for argininosuccinate lyase (ASL) has been isolated from C. reinhardtii and its nucleotide sequence determined. The gene spans a region of 7 kb, is interrupted by at least eleven introns and is associated with regions of highly repetitive DNA. Using this gene we have developed a nuclear transformation system whereby arginine-requiring mutants carrying lesions at the ASL gene locus are rescued to a wild-type phenotype. Analysis of transformants reveals variable patterns of integration of the cloned DNA into the nuclear genome and ASL activities comparable with wild-type cells, although the original lesions do not appear to be repaired. We are presently using this system to: i) construct an expression vector using the ASL gene promoter to express various drug resistance and reporter genes in C. reinhardtii; ii) screen for regions of the C. reinhardtii nuclear genome which promote autonomous replication of the transforming plasmid, and iii) develop a cosmid library for the rescue of double mutants using a shotgun transformation approach.

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EXPRESSION OF THE CHLOROPLAST psbB GENE IN *CHLAMYDOMONAS REINHARDTII* IS DEPENDENT ON A NUCLEAR GENE PRODUCT

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The psbB gene codes for an approximately 50 kD protein termed P5, that is one of the chlorophyll a-binding core polypeptides in Photosystem II. This protein is chloroplast-encoded, but as experiments with the mutant 222E have shown, its expression requires a nuclear gene product likely to act at the post-transcriptional level. Mutant 222E was isolated on acetate-containing media after mutagenesis and suicide-selection, and was characterized as a Photosystem II mutant by its high fluorescence phenotype. Genetic crosses showed the mutation to be nuclear in origin. Pulse-labeling of total proteins with ^{14}C-acetate demonstrated the absence of the psbB protein product. Northern blot analysis failed to detect any psbB message in the mutant and also suggested processing of the psbB message in wild-type cells. In vivo ^{32}P-UTP pulse-labeling of total RNA showed that the psbB message is transcribed at wild-type levels in the mutant. This suggests that a nuclear factor involved in psbB expression is acting post-transcriptionally, either mediating message stability or splicing.

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INFECTIOUS MEASLES VIRUS FROM CLONED cDNA

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The study of measles virus (MV) and of negative strand RNA viruses in general has been hampered by the lack of an experimental system for genetic manipulation. Here we describe a procedure for generating infectious MV from cloned MV cDNA. First we assembled a genetically marked DNA copy of the MV genome in plasmids, under the control of phage T3 or T7 promoters, allowing production of transcripts almost identical to the MV genome or antigenome. Incubation of these linearized plasmid DNAs with the appropriate phage polymerase and only two ribonucleoside triphosphates yielded committed transcription complexes. Microinjection of these complexes into the cytoplasm of helper cells which provide the proteins necessary for MV genome encapsidation and transcription/replication, reproducibly gave rise to lytic MVs. The transcripts of one of these viruses were analysed by sequencing after reverse transcription followed by DNA amplification, and found to contain the genetic tags. The described procedure permits to analyse a negative strand RNA virus with the same genetic tools previously applicable only to positive strand RNA viruses and retroviruses.

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TRANSLATION AND GLYCOSYLATION OF THE MEASLES VIRUS ENVELOPE PROTEINS

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The measles virus (MV) envelope contains two glycoproteins: fusion (F) protein, which causes fusion of cell membranes, and hemagglutinin (H), necessary for attachment to cellular receptors. The influence of the unusually long leader sequence (573 bases) on translation of monocistronic F transcripts was studied in a rabbit reticulocyte system. When the leader was deleted, efficiency of translation increased 5-10 times. When bicistronic transcripts containing the matrix (M) gene upstram of the F gene were used, F protein translation decreased only slightly. When only 431 bases of the M trailer sequence were left upstream of the F leader, an F protein with 329 additional amino acids at its amino terminus was obtained. We will investigate whether such a protein is produced *in vivo*. Moreover, the glycosylation of the F and H proteins of four defective MVs, derived from brains of patients who died from MV persistent infections, was compared with that of the MV vaccine strain Edmonston. As judged by the size of the products obtained after translation *in vitro* in presence of microsomal membranes, the glycosylation of all of the F and of three of the four H proteins derived from defective MVs was normal. In the only exception, the corresponding cDNA contained point mutations, resulting in the elimination of two of the five potential glycosylation sites.

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VECTORS FOR *IN VITRO* PRODUCTION OF POLYADENYLATED MEASLES VIRUS RNAs

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Measles virus (MV) infections are one of the major causes of infant death in third world countries, and lead to rare but fatal diseases of the central nervous system. We previously cloned and sequenced full length genes from lytic and persistent MVs, but a functional characterization of these genes requires their expression in eukaryotic cells. For this, a set of expression vectors containing all seven MV cDNAs was modified by shortening the vector sequences located upstream of the MV genes and inserting a polyA-polyT tract of 63 base pairs downstream of each gene. A high tendency for spontaneous partial elimination of the polyA-polyT tract from the plasmids was monitored. However, clones which maintained the original sequence were obtained. RNAs which contained a 5' cap and a poly-A tail, transcribed *in vitro* with T7 RNA polymerase, gave rise to proteins of the expected size in a rabbit reticulocyte lysate. We plan to directly introduce these RNAs into eukaryotic cells by electroporation and/or lipofection. This will allow to produce MV proteins in appropriate relative ratios by using pools of RNAs of different composition. This system will be used in conjunction with infectious genomic MV cDNA clones (see poster by Ballart et al.), to functionally study mutations either artificially constructed or naturally occuring in persistent MVs.

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MANY PERSISTENT MEASLES VIRUSES SHOW DRASTIC ALTERATIONS IN THE FUSION PROTEIN CYTOPLASMATIC DOMAIN

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In rare cases measles virus (MV) persists, causing lethal human diseases such as subacute sclerosing panencephalitis (SSPE). Previous sequencing of cloned MV genes from 3 SSPE cases revealed multiple mutations affecting all viral proteins at different degrees. Since the short carboxyl terminal cytoplasmatic domain (33 amino acids) of the MV envelope fusion (F) protein appeared consistently truncated, additional 8 SSPE cases were investigated for the presence of analogous defects, mostly by sequencing PCR amplified cDNA's of this region. Among all 11 cases analysed 4 showed different nucleotide substitutions giving rise to stops 8, 16, 19 and 24 codons prior to the normal stop, 2 showed 1 nucleotide deletion, shifting the reading frame 27 codons prior to the normal stop, 1 had the normal stop abolished and 1 revealed 4 aminoacid substitutions concentrated in the cytoplasmatic domain. Thus, most F proteins in SSPE cases, although well conserved over 94% of their length and therefore presumably active to mediate cell fusion, are drastically altered at the carboxyl terminus, probably limiting cell surface expression and virion formation.

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EXPRESSION OF HUMAN MxA PROTEIN IN TRANSFECTED MOUSE 3T3 CELLS CONFERS RESISTANCE TO INFLUENZA VIRUS AND TO VSV

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Resistance of mice to influenza virus is brought about by the action of the interferon-induced 72-kDa nuclear Mx1 protein. We have recently isolated and characterized the cDNA clones of two human Mx-related genes designated MxA and MxB. In contrast to murine Mx1, their protein products accumulate in the cytoplasm. To elucidate their antiviral potential we transfected Mx protein-deficient mouse 3T3 cells with mouse or human Mx cDNAs under the control of a constitutive promoter. Several independent stable cell lines were established which uniformly expressed high levels of the different Mx proteins. Cell lines expressing the nuclear murine Mx1 or the cytoplasmic human MxA protein acquired resistance to influenza virus. Surprisingly, MxA protein also conferred resistance to VSV, a rhabdovirus. However, Mx1 or MxA protein producing cell lines were sensitive to EMC virus, Mengovirus, HSV-1 and SFV. Transfected cells accumulating the human MxB protein in the cytoplasm were susceptible to all viruses mentioned above. Our results thus confirm the selective antiviral activity of mouse Mx1 protein towards influenza virus. We further conclude that the human MxA protein has a broader antiviral activity. Taking advantage of the Mx protein expressing cell lines, we currently examine the mechanism by which Mx proteins interfere with virus multiplication.

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ANTIVIRAL POTENTIALS OF MUTANT MURINE AND HUMAN Mx PROTEINS

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The murine influenza virus resistance locus Mx consists of two interferon-regulated transcription units, Mx1 and Mx2. The 72-kDa Mx1 protein is a nuclear protein. When expressed in transfected cells Mx1 protein confers selective resistance to influenza virus. Cytoplasmic forms of Mx1 protein carrying mutations in the nuclear transport signal failed to inhibit influenza and all other viruses tested, suggesting that the activity of Mx1 protein is restricted to the nucleus. The Mx2 gene is defective in all inbred mouse strains tested to date: an insertion of one nucleotide interrupts the open reading frame and destroys the coding capacity of Mx2 mRNA. Mx2 cDNA was repaired by site-directed mutagenesis and transfected into Mx protein-deficient mouse 3T3 cells. Transfected cells accumulated the 74-kDa Mx2 protein in the cytoplasm and thereby acquired resistance to vesicular stomatitis virus (VSV). The murine Mx2 protein thus resembles the cytoplasmic human MxA protein which inhibits the multiplication of both influenza virus and VSV. Since GTP-binding consensus elements are present in all Mx proteins, we speculate that Mx proteins act via a GTP-dependent biochemical activity. To test this, we currently express Mx proteins with single amino acid changes in the GTP-binding consensus elements and test transfected cells for acquired resistance to VSV and influenza virus.

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CLONING AND SEQUENCE ANALYSIS OF IFN-INDUCED HUMAN Mx PROTEINS REVEALS A PUTATIVE GTP-BINDING SITE.

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cDNA clones encoding two interferon(IFN)-induced human proteins, which are homologous to the murine Mx protein, have been obtained. The proteins of deduced M_r 75.5K (Mx1) and 72.5K (Mx2) are related antigenically, and share a 63% sequence homology. Both proteins have been translated in the reticulocyte system using mRNAs transcribed from the cDNAs. Sequence homology in this family of proteins is most pronounced at the NH_2-terminus, indicating that an active site might have been preserved in this region. In fact we have found that these proteins possess near their NH_2-terminus three consensus elements in proper spacing, which is a characteristic of nucleotide (GTP)-binding proteins. Mx proteins seem to belong to a new subgroup of GTP-binding proteins, comprising SRP 54K and docking proteins, involved in the vectorial transport/unfolding of proteins.

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THE MURINE INTERFERON-γ RECEPTOR GENE.

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Interferon-γ (IFN-γ) is produced by activated T-cells and exerts a variety of biological effects including antiviral activity, inhibition of cell growth and activation of macrophages. It modulates MHC antigen expression and probably plays a crucial role for regulating antigen presentation. IFN-γ exerts its effects through binding to a specific cell surface receptor. Recently the human and murine IFN-γ receptor (IFN-γR) have been cloned (Cell 55:273, 1988; PNAS, in press). Using murine IFN-γR cDNA probes several genomic clones were isolated from a genomic EMBL3 library. Restriction enzyme mapping revealed that the inserts represent a region of 28 kb that contains the complete single copy murine IFN-γR gene. Like its human counterpart (manuscript in preparation) the murine IFN-γR gene consists of seven exons and the membrane spanning region is encoded by exon 6.

To design novel models for investigating the physiological role of IFN- γ we plan to delete the murine IFN-γ gene *in vivo* using recently established strategies for gene targeting by homologous recombination. Two targeting vectors containing 2 and 4 kb of the murine IFN-γR gene respectively have been assembled. Both constructs contain exon 5 which has been disrupted by insertion of the neomycine resistance gene. These constructs are currently used for transfection of mouse embryonic stem cells. G-418 resistant clones are screened for homologous recombination using the PCR.

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COOPERATION OF MMTV ORF GENE IN EPITHELIAL CELL TRANSFORMATION

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The U3 region of the Mouse Mammary Tumor Virus LTR contains a large open reading frame (orf) potentially coding for a protein of 36 kd. This coding sequence is highly conserved in all MMTV strains but as yet the function of the protein is not known. Expression vectors of the open reading frame have been used to study its ability to transform an immortalized rabbit mammary cell line (RMC-SV-40). either alone or in cooperation with the ras, neu or myc oncogenes. Pools of cells transfected with either orf alone or orf & neu gave tumors of low growth potential after injection in nude mice. When we analysed the levels of transcription by S1 analysis, we found a positiv correlation between orf specific RNA and the tumorigenicity of different pools of cells. In double transfectants low levels of specific gene products seem to be sufficient for transformation to take place. On the other hand, high expression of ras, neu or myc never resulted in tumor formation.

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TISSUE SPECIFICITY OF MOUSE MAMMARY TUMOR VIRUS

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The BALB/cf/Cd substrain of mice, selected from a colony of BALB/c mice foster-nursed on C3H mothers, shows a 70 % incidence of kidney adenocarcinomas, whereas mammary tumors completely disappeared. A new horizontally transmitted provirus, MMTV-K, was isolated from a transplanted tumor.
DNA analyses of different organs and primary kidney lesions of BALB/cf/Cd mice did not reveal newly integrated proviruses. However, using the polymerase chain reaction (PCR), we detected MMTV-K DNA in tumors and in most tissues of BALB/cf/Cd animals (e.g. thymus, salivary glands, testis, male accessory glands, liver, kidney, spleen, mammary glands, ovaries, uterus). PCR is unable to distinguish if the virus is present in circulating cells or in the tissue itself. We therefore performed in situ hybridization experiments to identify the cells in which MMTV-K is transcriptionally active.
In parallel, we started analysing the behaviour of the complete MMTV of mammary and kidney origin in transgenic mice (in collaboration with Dr. E. Kolb, Zürich). Work in progress includes RNA expression, detection of proteins in the milk, presence of infectious particles as tested by foster-nursing, and tumor formation.

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COMPARISON OF ANTIGENIC STRUCTURES OF HEPATITIS A VIRIONS AND EMPTY CAPSIDS.

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Hepatitis A virus is a naked virus with a positive sense RNA genome. Infection with HAV of cell cultures is accompanied by production of various incomplete virus particles, namely empty capsids. Inactivated virus preparations from cell culture used as vaccines thus contain virus and incomplete virus particles. We investigated by competition assays with particle-specific polyclonal and monoclonal antibodies the potential of empty capsids to elicit a virus neutralizing response. Viral harvests were separated by banding in CsCl density gradients and sedimentation in sucrose density gradients. Convalescent sera were tested on separation profiles against polyclonal anti-virus and anti-empty capsid antisera; polyclonal antisera against neutralizing monoclonal antibodies and the latter against each other. The results show that empty capsids present on their surface immunodominant neutralizing antigenic structures of the virion. Empty capsids in vaccine preparations, therefore, should not only aid in eliciting an antiviral response but per se should be useful to induce a protective immune response.

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DETECTION OF A VIRUS ENCODED OR VIRUS INDUCED RNA BINDING FACTOR ASSOCIATED WITH ESTABLISHMENT OF PERSISTENT HEPATITIS A VIRUS INFECTION IN VITRO.

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Establishment of persistent infection is a regular result of hepatitis A virus (HAV) infection in cell cultures. Previous studies provided evidence that concomitantly with establishment of persistent infection production of viral RNA is down-regulated. This may be due to the activity of regulating factors. Using RNA/protein binding assays it was shown that, at the critical time during virus replication, a protein accumulates which interacts specifically with a distinct nucleotide sequence within the 5'terminal region of the HAV antigenome. The sequence CCUCAGAAAUUUUAAGAAAAUUUGU is complementary to a stretch of nucleotides in the 3'terminal non-coding region of HAV genomic RNA. A sequence with 83% similarity was found in the corresponding region of poliovirus type 1 RNA. The factor identified in HAV infected cells, however, could not be demonstrated in cells infected with poliovirus.

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Binding of empty MVM capsids to the 3'-terminal hairpin of MVM RF DNA

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During parvovirus infection the incoming single-stranded viral genome, about 5000 bases in length, is converted into a replicative form (RF) which can have two types of 3'-end, one covalently closed (hairpin), the other open (extended). With Aleutian disease virus (ADV) there is specific binding of viral and cellular proteins to the 3'-hairpin RF DNA, but not to the same sequence in its extended configuration. Thus DNA conformation and not just DNA sequence is important for these interactions. The 3'-terminal hairpin interacts with empty viral capsids. This interaction probably plays a role in the synthesis and subsequent encapsidation of viral progeny DNA. Similarly the 3'-end of the RF DNA of minute virus of mice (MVM) binds specifically to empty MVM capsids. Again the interaction is specific for the hairpin form. We are currently doing DNase footprinting to localize the DNA-binding sequence.

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MODULATION OF SIGNAL TRANSDUCTION BY POLYOMA MIDDLE-T IN A HEMATOPOIETIC CELL LINE

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Middle-T antigen (mT), one of the early gene products of polyoma virus, forms a stable complex with cellular tyrosine-specific kinases as e.g. pp60$^{c\text{-}src}$. Complex formation causes a 20-50 fold increase in kinase activity and is necessary but not sufficient for cell transformation by polyoma virus. We expressed mT in the bone marrow-derived established cell line FDC-P1 and investigated its influence on the growth factor requirement of these cells. Control FDC-P1 cells are strictly dependent on either GM-CSF or IL-3 for survival and growth while a subpopulation of mT-expressing cells was fully factor-independent. Factor-independent sublines produce low amounts of GM-CSF, but no IL-3. However, these cells are not dependent on GM-CSF secreted into the medium, as they are not inhibited by neutralizing anti-GM-CSF antibody.

We further investigated whether factor independence of these cells is mediated through constitutive activation of pp60$^{c\text{-}src}$. Overexpression of pp60$^{c\text{-}src}$ in FDC-P1 cells did not result in a phenotype similar to mT-expressing cells, suggesting that mT might alter the substrate specificity as well as the specific activity of pp60$^{c\text{-}src}$.

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Myristylation of pp60$^{c\text{-}src}$ is not required for complex formation with polyoma virus middle-T antigen

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Polyomavirus belongs to the group of the DNA tumor viruses. In various cell lines the expression of one of the three tumor antigens, middle-T antigen (mT), is sufficient to cause transformation. Polyoma mT interacts with several cellular proteins e.g. the cellular tyrosine kinase, pp60$^{c\text{-}src}$. Both mT and pp60$^{c\text{-}src}$ are localized in the plasma membrane, the former via a carboxyterminal stretch of 22 hydrophobic amino acids the latter via a myristate group attached to the N-terminal glycine. It has been shown that myristylation is essential for transformation and proper localization of a mutant retroviral form of pp60$^{c\text{-}src}$ (pp60$^{v\text{-}src}$).

We were interested in the question whether complex formation between pp60$^{c\text{-}src}$ and mT is affected using a mutant non-myristylated pp60$^{c\text{-}src}$. For this purpose we transfected 3T3 cells with either wt or a mutant chicken c-src gene in which the codon for glycine 2 has been converted to GCA (alanine).

Cell fractionation showed that the wt protein is found in the particulate (membrane) fraction while the mutant protein is present in the soluble fraction. However, complex formation of the mutant protein with mT is not affected and the complex is found in the particulate fraction. In wt and mutant c-src-expressing cell lines mT-bound pp60$^{c\text{-}src}$ is activated.

These results suggest that the hydrophobic membrane anchor of mT is sufficient for membrane binding of complexed pp60$^{c\text{-}src}$. It is unclear whether the complex is formed in the cytoplasm right after synthesis or after the two proteins have reached the plasma membrane individually.

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IMMEDIATE EARLY (IE) GENES OF HERPESVIRUSES ENCODING THE p180 FAMILY OF TRANSACTIVATOR PROTEINS

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Herpesvirus replication occurs in three distinct temporal phases named "alpha" or "immediate early" (IE), "beta" or "early" (E) and "gamma" or "late" (L), which are regulated by viral proteins acting mainly at the level of transcription. The best studied of these transactivator proteins is ICP4, an IE protein regulating the E phase of infection by herpes simplex virus 1 (HSV1). We have determined the entire nucleotide sequence of the corresponding IE gene of a porcine herpesvirus, pseudorabies virus (PRV), and part of an IE gene of bovine herpesvirus 1 (BHV1). Comparison with published sequences of varicella zoster virus (VZV) and equine herpesvirus 1 (EHV1) reveals that the encoded IE proteins share extensive sequence homology and justifies their grouping in a family named p180 (Phosphoproteins with approximate mol.wt.180,000; see also poster by Wirth et al.). Two regions of the PRV IE protein extending from amino acids 482 to 659 (region 2) and 959 to 1350 (region 4) exhibit 50-65 % identity with the cognate sequences and seem to be crucial for transactivation. We have studied transactivation by PRV and BHV1 gene products using CAT-assays. Regions 1, 3, and 5 of the p180 proteins had previously been thought to be nonhomologous. However, since sequences for five different viruses are available, additional regions of 30 - 45% identity have now been discovered.

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DETECTION OF HERPES SIMPLEX VIRUS IN CLINICAL SAMPLES BY THE POLYMERASE CHAIN REACTION

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We used the polymerase chain reaction to detect HSV-1 and HSV-2 in supernatants of infected cell cultures and in various tissues and body fluids of infected patients.

A region of the HSV-polymerase-gen was amplified in 30 PCR-cycles, using the primers MI1 and MI2 (M. Cao et al, J. invest. Dermatol. 82:391-392, 1989), yielding an amplimer of 90bp. After electrophoresis and Southern blotting to a nylon membrane, the amplification products were hybridized with an oligonucleotide probe (MI3, 5'-CAGGACTTTG TCCTCACCGC CGAACTGAGC AGACACCCGC-3') and the results were visualized by autoradiography. Sensitivity in detection of HSV increased from 100 infectious units to 0.1 IU, when the number of PCR-cycles was raised from 25 to 35.

PCR could be a valuable tool for detecting HSV in cerebrospinal fluid in cases of herpes encephalitis, where other methods normally fail to detect the virus. We intend to correlate the results of PCR with in situ hybridization and immunocytochemistry on consecutive tissue sections from parraffin embedded tissues.

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IMMEDIATE-EARLY GENE-PRODUCTS OF BOVINE HERPESVIRUS 1 (BHV-1)

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We have previously mapped BHV-1 transcripts expressed in a temporal cascade during lytic infection. Among 54 transcripts we could identify four as immediate-early (IE) and ten as late using the metabolic inhibitors cycloheximide and cytosine arabinoside. Three major IE RNAs (1.7, 2.9 and 4.2 kb) and one minor IE RNA (about 7 kb) originated from genome regions in or around the inverted repeat (IR) sequence of BHV-1. Three different BHV-1 subtypes (Jura, K22 and N569) produced similar IE RNAs with slight size differences. The transcripts were analyzed using northern blots, S1 nuclease, primer extension, cDNA cloning and sequencing. The 1.7 kb RNA is transcribed from the IR sequence part proximal to the unique short (US) region, the 3' end being situated around the IR/US junction. The 4.2 and 2.9 kb RNAs are transcribed in the other direction. Both share the same promoter and arise by alternative splicing and termination. The 4.2 kb RNA terminates within the IR sequence; on the other hand the 2.9 kb RNA extends into the unique long sequence. Whereas the mRNA level of the 1.7 kb RNA steadily increased during lytic infection, levels of the 2.9 and 4.2 kb RNA decreased after two hours post infection. Radioimmunoprecipitation and autoradiography of [35S]methionine or [32P]orthophosphate labeled IE proteins showed three major phosphoproteins (approx. 180, 135 and 50 kDa). Further structural and functional analysis of the IE gene-products and comparison to other herpesviruses is in progress (part of it will be presented by Schwyzer et al).

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COMPARATIVE BIOLOGICAL AND GENOMIC ANALYSES OF A BOVINE ISOLATE OF EQUINE HERPESVIRUS 1

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A strong species-specificity is attributed to most members of the herpesvirus group. An unexpected crossing of the species barrier, however, has been observed in equine herpesvirus 1 (EHV1). Hitherto no significant differences between common equine isolates and non-equid isolates have been detected.

In the present study we analysed the behaviour of a bovine EHV1 isolate in comparison with reference virus strains during passage in heterologous cell cultures. Cross-neutralization tests were carried out to detect possible antigenic relationships between EHV1 and bovine herpesvirus 1 (BHV1). Finally, we looked for genomic alterations during serial passage of the EHV1 strains in bovine cells by means of restriction enzyme analysis.

The EHV1 strains proved to replicate as well in bovine as in equine cells. No obvious antigenic relationship between EHV1 and BHV1 was demonstrable. Serial passage of the EHV1 strains in bovine cells induced only minor genomic alterations. The growth characteristics and the relative genome stability of EHV1 in bovine cells might be indicative of the abilitiy to cross the species barrier.

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CONSTRUCTION AND APPLICATION OF A COSMID LIBRARY OF THE GENOME OF WILD TYPE AFRICAN SWINE FEVER VIRUS.

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The genome of African Swine Fever virus (ASFV) consists of a large double stranded DNA molecule (150-180 kbp) with covalently closed ends. Wild type (wt) virus strains do not replicate in cell cultures but have to be propagated either in the host (pig) or in cultivated macrophages. We collected erythrocytes (Ec) from viraemic pigs (Ec are closely associated with virus particles). Virus particles were purified by ultracentrifugation, viral DNA was extracted, partially digested with Sau3A and cloned into cosmids using the BamHI site of pHC79. At present, approximately 70% of the wt ASFV genome has been cloned in cosmids as 4 overlapping fragments of between 30 and 60 kbp. The relative genomic positions of the cosmid-cloned viral DNA fragments were mapped by hybridization with radioactively labelled probes of ASFV DNA. A highly conserved, ASFV specific region within cosCM 34 was identified and sequenced. From this DNA sequence, oligonucleotide primers were synthesized and used in a polymerase chain reaction for the detection of ASFV in organs. In addition, subclones derived from the cosmid-cloned viral DNA currently are being used to construct recombinants between cell culture adapted- and wt ASFV.

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Purification and structural characterisation of mouse U7 snRNPs involved in 3' processing of histone pre-mRNAs.

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The U7 snRNP is an essential cofactor of histone RNA 3' processing. Its function is mediated by basepairing between the free 5' end of U7 RNA and a conserved sequence element in the 3' spacer of histone pre-mRNA. U7 RNA contains the 2,2,7-trimethyl guanosine (m3G) cap structure typical of most U RNAs. Therefore U7 snRNPs copurify with other snRNPs on an affinity column of anti-m3G antibodies. Functional U7 snRNPs can then be separated from the bulk of the more abundant (U1-U6) snRNPs by Mono Q anion exchange chromatography. An additional separation step should yield essentially pure U7 snRNPs.

Another purification strategy makes use of an oligonucleotide complementary to the 5' end of U7 RNA linked to a gel matrix via biotin-streptavidin. Because this affinity ligand is similar to histone pre-mRNA, it may also bind other cofactors of the processing apparatus which may associate with U7 snRNPs only temporarily.

The aim of this study is to characterize the protein composition of U7 snRNPs and to provide a basis for the isolation of cDNA clones for potential U7-specific proteins.

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Interaction of snRNP and non-snRNP factors with histone pre-mRNA sequences during 3' processing in vitro.

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Histone-specific RNA 3' processing is controlled by two short cis-acting sequence elements in the pre-mRNA (hairpin loop and spacer elements) and at least three trans-acting factors: the U7 snRNP, a hairpin binding factor and a heat-labile activity. To characterise these factors, we have fractionated nuclear extracts by standard biochemical separation techniques and affinity chromatography. Interactions of these partially purified components with histone pre-mRNA have been studied in vitro by native gel retardation assays, UV cross-linking between RNA and protein and northwestern blotting. We have also observed specific interactions with DNA oligonucleotides corresponding to different parts of the pre-mRNA. The same techniques have also been applied to study the interactions between U7 RNA and potential U7-specific snRNP proteins. Some of the proteins revealed by these methods are good candidates for specific constituents of the processing machinery and further purification may lead to their structural characterisation and to the the isolation of cDNA clones.

In addition, we have begun to reconstitute U7 snRNPs in vivo by injecting synthetic U7 RNA into Xenopus oocytes. This approach should allow us to study the assembly, structure and function of the U7 snRNP.

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EVIDENCE FOR THE EXISTENCE OF SPLICING AND PROCESSING FACTORS SPECIFIC FOR THE GENES OF NUCLEAR ENCODED MITOCHONDRIAL PROTEINS

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As a continuation of the preparatory work on the existence of splicing factors specific for the introns of the genes of nuclear encoded mitochondrial proteins, we have looked at the other consensus sequences, known as necessary for the splicing and processing. This search resulted in several variants and some new consensus sequences:
a) consensus sequence at the 3'pre-mRNA end. Total of 35 3'pre-mRNA ends has been statistically evaluated and resulted in the following consensus: TTTT.T.T.TTTCAATAAA...T...T. This finding is in good agreement with the already published experimental evidence.
b) consensus sequence around initiator methionine Total of 67 cDNA 5'ends have been statistically evaluated and resulted in the consensus sequence: G.CACCATGGC, showing differences at several positions to the known consensus sequence CCPCCATGG.

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FACTORS INVOLVED IN THE ASSEMBLY OF SPLICING COMPLEXES

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The removal of introns from nuclear messenger RNA precursors (pre-mRNA) occurs in large multicomponent complexes (spliceosomes). These complexes are assembled in a stepwise fashion which involves the sequential binding of the major U-type snRNPs to the pre-mRNA. In addition, protein factors participate in the assembly process. We are purifying these factors by biochemical means and could identify two fractions (SF1 and SF3) which are required for the formation of a pre-splicing complex and thus for the complete splicing reaction. The use of synthetic oligoribonucleotides, carrying sequences characteristic for the 3' end of introns, led to the identification of (at least) two components in SF3-containing fractions that specifically bind to the oligoribonucleotide and to pre-mRNAs. These factors have been separated chromatographically and results will be presented concerning their involvement in the assembly of splicing complexes.

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FACTORS INVOLVED IN THE SPLICING OF MESSENGER RNA PRECURSORS

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The removal of introns from nuclear pre-mRNA can be separated into multiple steps in vitro. Following the assembly of the spliceosome (which contains the major U-type snRNPs) the pre-mRNA is cleaved at the 5' splice site, a reaction that gives rise to the splicing intermediates. The RNA is then cleaved at the 3' splice site, the exons are ligated, and the intron is released. We are studying these reactions in chromatographic fractions prepared from HeLa cell nuclei with special interest in the protein factors that participate in the reaction. In addition to snRNPs, SF1 and SF3 are involved in early processes of splicing complex formation. These factors, as well as SF2 are required to assemble the spliceosome. The splicing reaction can then be initiated by addition of a fourth protein fraction, SF4. Thus, SF4 represents a factor that is involved in the first cleavage and ligation event. The purification of this factor and its characterization will be presented.

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HIGH YIELD RNA-AFFINITY PURIFICATION OF IRF, A SPECIFIC POST-TRANSCRIPTIONAL REGULATORY PROTEIN

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IRF is a cytoplasmic mRNA-binding protein which regulates the expression of both human transferrin receptor (hTR) and ferritin by modulating their mRNA stability and translation, respectively. This regulation requires specific palindromic sequence elements (IRE) located in the 3' untranslated region of the hTR, and in the 5' untranslated region of the ferritin mRNA. We have purified IRF from human placenta by taking advantage of its specific RNA-binding properties. This affinity purification procedure has the potential of being generally applicable for isolating RNA-binding proteins. In vitro transcribed polyadenylated RNA is bound to poly(U)-Sepharose and incubated with cytoplasmic proteins prepurified on a heparin column. Specifically adsorbed proteins are recovered in high yield and purity from the affinity matrix by high salt elution. Affinity purified IRF migrates as a 95/100 kDa doublet on SDS-PAGE. The RNA-binding activity of IRF can be activated in vitro upon reduction.

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RAS ONCOGENES STABILIZE CALCIUM INDUCED IL-3 mRNA BY A POSTTRANSCRIPTIONAL MECHANISM

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Previous work from our laboratory has shown that IL-3 expression in mast cells is regulated posttranscriptionally and can be induced by calcium ions which stabilize IL-3 mRNA. Expression of IL-3 in PB-3c normal mastocytes can also be induced by oncogenic transformation, a process requiring a ras oncogene. It is not known whether oncogenic activation of IL-3 takes similarly place by a posttranscriptional mechanism. In this report, we examined whether various human ras alleles synergize with calcium-induced induction of IL-3 and GM-CSF. Use was made of dexamethasone inducible MMTV-LTR constructs bearing either EJ-Ha-ras, activated N-ras or the N-ras protooncogene. Activated ras alleles amplified the calcium effect, the normal allele did not. Experiments using actinomycin D revealed that the ras effect was posttranscriptional: the half life of IL-3 mRNA in the absence of oncogene expression was about 15 min, and was prolonged to over 2 hr by inducing the oncogene. This is, to our knowledge, the first report that the ras oncogene affects a posttranscriptional mechanism of gene expression.

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ROLE OF A mRNA-BINDING PROTEIN IN IRON HOMEOSTASIS

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A post-transcriptional regulatory protein, termed iron regulatory factor (IRF), that binds specifically to the iron-responsive elements of ferritin and transferrin receptor mRNA, has recently been identified in the cytoplasm of human and mouse cells. Binding of this factor inhibits ferritin translation and correlates with an increase of TR mRNA stability. We found that this mRNA-binding protein is conserved among both vertebrate species and insects. In the mouse, IRF appeared to be expressed in all tissues analysed, suggesting that iron homeostasis is ubiquitously controlled by post-transcriptional regulatory mechanisms. Invariantly, the mRNA-binding activity of IRF was modulated by intracellular iron levels and involved an oxido-reductive process. In parallel with levels of TR mRNA, IRF activity was strongly induced during growth stimulation but did not change throughout the cell cycle. This result suggests that high expression of TR in cell proliferation is at least in part the result of IRF activation.

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MODULATION IN VIVO OF THE β_1- AND β_2-ADRENERGIC RECEPTOR mRNA LEVEL IN LEAN AND OBESE ZUCKER (fa/fa) RAT BROWN ADIPOSE TISSUE BY THE THERMOGENIC β-ADRENERGIC AGONIST Ro 16-8714

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The mRNA of both the β_1- and β_2-adrenergic receptor (AR) subtypes are found to be expressed in rat brown adipose tissue (BAT), the β_1-AR being the more abundant. The sizes of the two subtypes are 2.9 and 2.3 kb, respectively. Treatment of lean and obese (fa/fa) Zucker rats with the thermogenic β-agonist Ro 16-8714 induces in the obese animal a rise in the β-AR receptor number after 30 h followed by a return to basal value at 48 h. It decreases the β_1-AR mRNA 1.6-fold in obese rat and the β_2-AR mRNA 1.9- and 4.2-fold in lean and obese rats, respectively. The same treatment has no detectable effect on the β_1- and β_2-AR mRNA of white adipose tissue. The results show that a β-agonist reacting selectively with the atypical β-AR of the BAT induces a down regulation of the β_1- and β_2-AR mRNA and that this down regulation is amplified in obese animals as compared to lean.

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ANTI-SENSE RNA PRODUCED FROM A POL III EXPRESSION VECTOR INHIBITS GENE EXPRESSION IN *XENOPUS* EMBRYOS

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An inverted gene fragment has been inserted into the Adeno viral VAI gene.This vector injected into *Xenopus* zygotes is amplified during cleavages and produces, after mid-blastula transition, large amounts of RNA carrying an anti-sense segment. The anti-sense RNA/mRNA hybrids are rapidly degraded. Expression of the cytokeratin 8 gene (Franz & Franke 1986, Proc. Natl. Acad. Sci. USA 83, 6475) is completely inhibited up to developmental stage 25. Thus, a gene that is expressed at high levels can be inhibited during a crucial phase of development in which many determination and early differentiation steps take place. Anti-sense inhibition of the homeotic gene hox1A (Harvey et al., 1986 : EMBO J. 5, 1237) indeed causes heavy malformations in tail, skeletal muscle and intestinal tract.

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S.CEREVISIAE TRANSLATION INITIATION FACTOR 4E

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One of the first steps in the initiation of protein synthesis involves binding of the translation initiation factor 4E (eIF-4E) to the cap structure at the 5' end of eukaryotic mRNA. To study the function of eIF-4E in translation initiation, we established an eIF-4E-dependent crude cell-free yeast extract, eIF-4E affinity chromatography, and eIF-4E immunoprecipitation and phosphorylation assays. Experiments with the eIF-4E-dependent cell-free extract show that (i) certain mRNAs do not require the initiation factor eIF-4E for translation and (ii) certain mRNAs initiate translation by a cap-independent internal pathway. Like the mammalian eIF-4E (which forms a complex with the proteins p220 and eIF-4A, called eIF-4F) the yeast eIF-4E also copurifies on cap-analogue column with two other proteins, one of 150 kD and one of 18 kD. To verify the proposed complex formation of these three proteins, we performed immunoprecipitation studies with a monoclonal anti-yeast-eIF-4E antibody coupled to Protein G-Sepharose. The data show that a complex analogous the mammalian eIF-4E also exists in yeast. Finally, we present data on the phosphorylation of yeast eIF-4E in vitro and in vivo. The possible regulation of eIF-4E activity by phosphorylation will be discussed.

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ANALYSIS OF THE TIF1 AND TIF2 GENES CODING FOR THE TRANSLATION INITIATION FACTOR 4A FROM THE YEAST S. CEREVISIAE.

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The eukaryotic initiation factor 4A from yeast (eIF-4A$_Y$) is encoded by two genes TIF1 and TIF2 which code for exactly the same protein but have highly divergent 5' and 3' flanking sequences. This suggests that the two genes are regulated differently, although inactivation of either gene has no effect on cell growth. Inactivation of both genes is lethal. The expression of both genes is currently investigated.

We have developed a conditional system, where the TIF1 gene is under the control of the hybrid CYC1-GAL promoter. Cells having this TIF1 gene as the only source of eIF-4A$_Y$ can no longer grow on glucose medium. We have used this system to isolate mutants in the TIF1 gene after random chemical mutagenesis and to analyze some site-directed mutants of three highly conserved domains of a new family of proteins (D-E-A-D proteins). Up to now, we have isolated leaky, lethal and a conditional mutant. These mutants will also be analyzed in in vitro translation and helicase assay systems and will then be used to isolate suppressors.

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NON-ORTHODOX CHROMATINS

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Bacteria, Archaebacteria, Dinoflagellates and others do not dispose of classical histones. Other DNA-binding proteins are present in about 10 smaller amounts. Are these involved in building compactosomes similar to nucleosomes?

On the above material it will be shown by immunocytochemistry that they are not always located with the bulk DNA but rather where transcription occurs. In vitro experiments with histones to DNA at ratios from 1:10 to 10:10 have shown that, when above 5:10, the resulting chromatin can be gelled through crosslinking with aldehydes. By this, the chromatin becomes resistant to aggregation during dehydration used in classical embedding procedures for electron microscopy. Only natural histone-chromatin is insensitive, all the others investigated ones aggregate.

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THE FATE OF NUCLEOSOMES DURING TRANSCRIPTION IN SACCHAROMYCES CEREVISIAE.

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The DNA of all eukaryotic cells is densely packaged by histone proteins in a linear array of nucleosomes. Since the histones might interfere with the RNA-polymerases, nucleosomes might be stable, unstable or they might dissociate during transcription. To investigate whether the fate of nucleosomes depends on (i) the rate of transcription or (ii) on the DNA sequence or (iii) on the transcription machinery, we construct genefusions in yeast *Saccharomyces cerevisiae* and study their chromatin structures in presence or absence of transcription. One construct (GAL-RIBURA) contains fragments of a ribosomal-RNA-gene of *Dictyostelium discoideum* fused to the coding region of a housekeeping gene (URA3). Transcription is initiated from the GAL1-10 inducible promoter. Preliminary results indicate that nucleosomes on the URA3 part remain stable while nucleosomes on the rRNA-part disintegrate during transcription.

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SHUTTLE MINICHROMOSOMES TO STUDY CHROMATIN STRUCTURES IN SACCHAROMYCES CEREVISIAE AND SCHIZOSACCHAROMYCES POMBE.

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We ask whether there are structural features in chromatin which are conserved amoung evolutionary diverged species - and hence might have a general significance - and whether there are structural features which are unique to an inidividual organism - and hence might be species specific. We therefore study how the same DNA sequence is packaged into chromatin structures by the two different organisms .

Using *DNA from S. c.*, plasmids were constructed which exist as extrachromosomal minichromosomes in S. c. and in S. p. The appearance of a nucleosomal repeat after nuclease digestion indicated that the plasmids were packaged into nucleosomes in both organisms. However, mapping of the nuclease cutting sites showed that nucleosomes were positioned in S.c. but randomly arranged in S. p.. A *DNA-sequence from S.p. (ADE6 gene)* showed precisely positioned nucleosomes in the 5'end both, when it was located in the chromosome or in a minichromosome. The sequence *per se* is not a sufficient determinant of the chromatin structure.

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STUDIES ON THE NEWLY ASSEMBLED CHROMATIN DURING SV40 DNA REPLICATION

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Simian virus 40 (SV40) DNA is associated with histones forming a minichromosome which shows similar properties like host cell chromatin. We study the newly replicated chromatin especially concerning the presence or absence of histone H1. We examine in replicating molecules the distribution of nucleosomal structures under destabilizing conditions which allow to distinguish between nucleosomes depleted of histone H1 and intact nucleosomes (Conconi et al., J. Mol. Biol., 178, 920, 1984).

Under these conditions we observed a lower proportion of nucleosomal DNA in the newly replicated (daughter) DNA segments compared to the prereplicative (parental) ones. The results therefore suggest indirectly that the newly replicated chromatin possibly lacks histone H1.

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REGULATION OF RIBOSOMAL CHROMATIN TRANSCRIPTION

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In mouse (and other organisms) transcribing ribosomal RNA genes are devoid of nucleosomes, whereas the inactive gene copies appear to be folded in a typical chromatin structure. A ribosomal RNA gene copy is either in its active or inactive state (Conconi et al., Cell 57, 753, 1989). The relative amounts of active and inactive copies can be determined by crosslinking of the DNA in chromatin, since the active copies take up significantly more psoralen than the inactive ones. In the adult mouse, liver cells contain 45%, pancreas 35% and spleen and kidney about 20% of active gene copies. These numbers correlate to the incorporation rate in the presence of α-amanitin of ribonucleoside triphosphates during transcription elongation in isolated nuclei (run-on experiments). These results indicate that ribosmal DNA gene transcription is regulated by modulating the number of active, respectively inactive gene copies.

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ENERGETICS OF DNA·STRAND EXCHANGE REACTION

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Purified RecA protein promotes in vitro all crucial steps of the recombination reaction i.e. recognition of homology between interacting DNA molecules followed by DNA strand exchange and separation of recombinant DNA molecules. In the process of such sequential reactions RecA hydrolyzes ATP and it has been proposed that the ATPase activity of RecA is necessary for the process of breaking of the original base-pairing and reformation of the new ones. We demonstrate here that RecA mediated strand exchange is independent of ATP hydrolysis. Polarity of strand exchange is also unchanged when instead, of ATP, its non hydrolyzable analog ATPγS is used. We show that the apparent ATP dependence of the recombination reaction is limited to post-exchange stages of the reaction. Low DNA affinity state of RecA protomers induced after ATP hydrolysis is necessary for the dissociation of RecA-DNA complexes at the end of the reaction. This dissociation is required for the release of recombinant DNA molecules from the complexes they formed with RecA and for resetting of RecA protomers for another round of the recombination reaction.

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ILLEGITIMATE RECOMBINATION IN THE FISSION YEAST SCHIZOSACCHAROMYCES POMBE.

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The behaviour of circular non ars plasmids in the fission yeast was studied. Every circular molecule introduced by transformation is able to replicate. Plasmid genome interactions were mainly performed by homologous recombination. However, if cross-hybridizing DNA sequences were diminished by deleting the corresponding sequences on the genome of the recipient strain 5% illegitimate recombination events were obtained. Genetic analysis were difficult because the recombinants were mitotically and meiotically unstable. Molecular analysis showed that 4/13 recombinants were integrated at the same target site. We detected at both interacting partners small and large DNA rearrangements. The recombination target sites were characterized by: Z-DNA structures, frequently small repeats from 3 to 6 bps or less frequently bigger repeats from 6 to 14 bps and additionally big polynucleotides up to 20 bps in arow were found. We also observed small homologies 3 to 5 bps between the interacting partners. Factors involved in this recombination events were only speculative.

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MOLECULAR ANALYSIS OF RECOMBINATION AT ADE6 OF SCHIZOSACCHAROMYCES POMBE

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We use the ade6 gene with its recombinational hot-spot mutation ade6-M26 to study homologous recombination during meiosis in fission yeast. M26 is a point mutation in the 5' region of the gene (Szankasi et al, 1988, JMB 204, 917), and shows a ten-fold increased formation of prototrophic spores in two factor crosses. Its disparity (in favour of conversion to wild-type) and its two-sided effect (Gutz 1971, Genetics 69, 317) led to the assumption, that the mutation acts as an initiation site for gene conversion. An extended genetic analysis of the gene with two factor crosses and flanking markers showed, that a region within the gene but in close proximity to its 5' end exists which exhibits the highest conversion frequencies. The mutation M26 lies within this region and seems to stimulate the naturally occurring processes. The interpretation of the genetic data that the high conversion end corresponds to an initiation site for recombination fits with the physical analysis of the conversion events: We used five restriction site polymorphisms to determine the length and the endpoints of conversion tracts in various crosses. The mean length of the tracts is dependent on the position of the crossed alleles, suggesting a fixed region in the gene, where the events are initiated. The great majority of the tracts is continuous, and post meiotic segregation (PMS) of restriction sites is detected only in a few cases.

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Site-directed Mutagenesis of the Nitrate Reductase (NR) Gene nia-2 in Plants.

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We would like to establish a model system for site-directed mutagenesis in plants. Since gene replacement (gene targeting) could be obtained in plants only at low frequencies , there is need to apply a direct selection system to monitor gene disruption. The NR-gene represents a suitable model . Plants that lack a functional NR-gene loose the capacity to grow on a media containing nitrate as a sole nitrogen source. NR also uses chlorate as a substrate and reduces it to the highly toxic chlorite . As plant material we used a haploid Nicotiana tabacum strain possessing only one functional NR-gene, nia-2. We attempted to disrupt it by a kanamycin resistance gene flanked by sequences of NR. Transformants selected on kanamycin were further screened for their ability to grow on a chlorate containing medium and their inability to grow on a medium containing nitrate. Molecular analysis are in progress.

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Self-replicating vectors as a tool for gene targeting in plants.

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Gene targeting is the directed integration of foreign DNA into a predicted genomic locus via homologous recombination. It requires an overlapping region of homology between the incoming and the target sequence. The gene targeting frequency can be estimated by the efficiency of the repair of a nonfunctional drug-resistance gene integrated into a nuclear genome. In plants gene targeting was achieved but at low frequency. In order to increase this frequency the noninfective DNA A component of Cassava Latent Virus (CLV) was used as a self-replicating, extrachromosomaly persisting unit. Here we report results of transformation experiments with protoplasts of transgenic tobacco strains containing the nonfunctional fragment of the drug-resistance gene using the viral vectors carrying the corresponding overlapping region of the gene. Homologous recombination between the viral replicon and the the chromosomal DNA should lead to the restoration of the gene structure.

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RETROVIRUS VECTOR FOR TRANSFER AND EXPRESSION OF THERAPEUTICALLY USEFUL LEVELS OF THE HUMAN β–GLOBIN GENE

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Gene therapy is being considered as a treatment for several diseases of the hematopoietic system , including β-thalassemia. At present, the major obstacles to this approach are inability to show efficient transfer of genetic material into pluripotent hematopoietic stem cells of animals and to obtain therapeutically useful levels of expression from the transfered human β-globin gene. Retrovirus vectors are the most promising means for gene transfer and attempts to transduce the human β-globin gene into murine bone marrow have demonstrated the difficulty in obtaining animals which show significant expression of transduced gene. Recently , the addition of 23 kb of sequences located 50kb upstream of the β-globin gene (LAR) led to high level, position independent expression of the human β-globin gene (Cell 51, p975). We have produced a retrovirus vector from the amphotropic packaging cell line PA317 , at a virus titer of 10^4 cfu/ml. The vector contains between two LTR's, a 2.0 kb human β-globin gene, a 2.7 kb LAR fragment and the NEO gene. Analysis of MEL cells infected with this virus showed that the level of expression of the introduced human β-globin gene is independent of the site of integration and at a level equal to that of the endogenous mouse β-globin gene.

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The Structure of Neutral Protease from *Bacillus cereus* at 2Å Resolution.

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Neutral protease (NP) is an extracellular metallo-endopeptidase, which hydrolyses the peptide chain at the imino side of aromatic and hydrophobic residues. The protein was isolated, purified and its amino acid sequence was determined by Sidler et al.(1986, *Biol. Chem. Hoppe-Seyler*,**367**,643-657.) They kindly provided the protein for crystallographic studies. NP was crystallized and the structure (3Å) was determined by molecular replacement using thermolysin (TLN) as a trial model (Pauptit et al. 1988, *J. Mol. Biol.* **199**,525-537).

2Å data were collected using synchrotron radiation at DESY-Hamburg, and the structure is now refined to a R-factor of 17.5%. Although NP shows 73% sequence identity with TLN, it is about 20° less thermostable. The refined model can now be compared with TLN with the hope of analyzing those features that may affect thermostability.

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Refined Crystal Structure of IGP Synthase : PRA Isomerase from *E.coli* at 2.0 Å Resolution.

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The crystal structure of indole glycerolphosphate synthase : N-5'-(phosphoribosyl)-anthranilate isomerase (IGPS:PRAI) from *E. coli* was determined at 2.8 Å resolution by multiple isomorphous replacement (J.P. Priestle et al., Proc. Natl. Acad. Sci. USA **84**, 5690-5694). A high resolution data set was collected by using synchrotron radiation at DESY, Hamburg. The structure has been refined by combined usage of the least-squares refinement program TNT and the molecular dynamics program X-PLOR resulting in a current model which has an R-factor of 20 % at 2.0 Å resolution. This bifunctional enzyme consists of two β-barrel domains which function as independent units. Details on the overall structure, the active site and interdomain contacts (between IGPS and PRAI) will be presented on the poster.

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X-RAY STUDIES ON MUTANTS & INHIBITOR COMPLEXES OF ASPARTATE AMINOTRANSFERASE FROM *E.COLI*

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W.t. aspartate aminotransferase (AATase) as well as mutant enzymes (V39L and K258H) from *E.coli* have been co-crystallized first with the inhibitor maleate from PEG 4000 at pH 7.5 and secondly without inhibitor from 45 % saturated ammonium sulfate. In both cases the space group and cell dimensions have been determined to be $P2_1$ and a=87 Å, b=80 Å, c=90 Å, β=119°. Several intensity data sets from mutant and w.t. crystals have been collected on area detectors to resolution limits between 2.9 Å and 2.4 Å . The structure of the V39L mutant has been solved by molecular replacement using a modified chicken mitochondrial AATase structure, in which the side chains were exchanged to give the *E.coli* w.t. sequence. W.t. AATase structure, soaked with the inhibitor 2-methyl aspartate, has been interpreted using a difference fourier map to 2.8 Å . Refinement is in progress. W.t. AATase and the K258H mutant enzyme, crystallized from saturated ammonium sulfate, have been solved by molecular replacement using the refined V39L mutant coordinates. The V39L mutant structure is of special interest since the enzyme shows higher activity than the w.t. enzyme for both natural and aromatic substrates.

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STRUCTURAL CHARACTERIZATION OF THE MITOCHONDRIAL CREATINE KINASE

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Mitochondrial creatine kinase, the membrane bound isozyme of the creatine kinase family, isolated from chicken hearts was reported to be a globular, octameric protein having a molecular weight of 340 kD (Schlegel et. al., 1988). Conventional electron microscopic investigations revealed a square shaped projection of the molecule with a central cavity when negative staining technique was used (Schnyder et. al., 1988). Molecules after freeze-drying and heavy-metal shadowing at very low specimen temperature (-250°C) under ultra-high vacuum($\leq 10^{-9}$mbar) showed a cross-like depression on the molecule surface. Computational image processing of individual octamers revealed only one view. Any search after a second view failed so far, leading to the assumption that the molecule consists of a cube-like entity with identical faces. The formation of linear aggregates under certain preparation conditions attributed, however, for different molecule faces. This suggests that the octamer has at least two sides which exhibit different physico-chemical properties. To get more insight, the enzyme was crystallized and the crystals which diffracted up to 3.2 Å showed two types of tetragonal unit cells. Electron microscopic studies of heavy metal stained crystals as well as metal replicas after freeze-fracturing of crystals in mother liquor confirmed the arrangement of the octamer in the crystal lattice. Supported by SNF grant No. 31-26384.89.

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COMPARISON OF THE ANTENNAPEDIA HOMEODOMAIN STRUCTURE WITH PROCARYOTIC REPRESSOR PROTEINS.

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The three-dimensional structure of the Antennapedia homeodomain from Drosophila was determined in aqueous solution by ^{1}H nuclear magnetic resonance (NMR) spectroscopy (Qian et al., Cell, 1989 in press). The structure comprises a helix from residues 10-21, a helix-turn-helix motif from residues 28-52, and a somewhat flexible fourth helix comprising the residues 53-59, which essentially forms an extension of the helix 42-52. The comparison with the procaryotic repressor proteins for which a three-dimensional structure has previously been determined by X-ray crystallography or by NMR (trp-, lac-, λ- and 434-repressor, CAP) shows that the helix-turn-helix motives in all these proteins and in the Antp homeodomain are nearly identical. Nonetheless, the individual proteins represent a variety of different molecular architectures. Overall, the comparative studies support the hypothesis that the helix 42-52 in the Antp homeodomain is the recognition helix, and that the helix 53-59 might also be involved in direct contacts with the DNA.

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BIOSYNTHETICALLY DIRECTED FRACTIONAL ^{13}C-LABELING FOR NMR ASSIGNMENTS IN PROTEINS.

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A new isotope-labeling strategy for biosynthetic proteins has been developped. The organisms used to express the protein are grown on a minimal medium containing a mixture of 10% [$^{13}C_6$]-glucose and 90% glucose with natural isotope distribution as the sole carbon source. The resulting characteristic ^{13}C labeling patterns are investigated by analysis of the cross peak fine structures in the [^{13}C,^{1}H]-COSY spectra. This method allows, for example, to obtain stereospecific assignments of the diastereotopic methyl groups in valine and leucine, to distinguish unam-biguously between the methyl groups of Ala, Thr and Ile, and to classify the 20 common amino acid residues into 4 groups on the basis of the ^{13}C-^{13}C scalar coupling fine structure at the C^{α} position.

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STRUCTURAL STUDIES ON THE LINKER POLYPEPTIDES AND B-PHYCOERYTHRIN γ-SUBUNITS IN THE PHYCOBILIPROTEIN COMPLEXES OF THE RED ALGA *PORPHYRIDIUM CRUENTUM.*

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As with cyanobacteria, the red alga *Porphyridium cruentum* contains large extramembraneous light-harvesting antennae known as phycobilisomes. These phycobilisomes are relatively large (up to $15 * 10^6$ Da) and their shape has been described as a large semi-ellipsoid through electron micrographs. The assignment however of the different linker - polypeptides to the various phycobilisome subcomplexes, especially to the core complexes, is not exactly known. Phycobilisomes of *Porphyridium cruentum* were prepared by a modified method and phycobiliprotein-complexes were isolated by ion-exchange chromatography on DEAE - Fractogel. The stoichiometric content of allophycocyanin, R - phycocyanin, b - phycoerythrin and the hexameric B - phycoerythrin in the phycobilisomes was determined. The size of the different phycobiliprotein complexes, the content of linker polypeptides and the spectroscopic properties were determined. γ-subunits were isolated and peptide fragments were prepared by chemical and enzymic cleavages to give a partial determination of their amino-acid sequences.

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Large Scale Isolation of Linker Polypeptides from Phycobilisomes of the Cyanobacterium *Mastigocladus laminosus*

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Phycobilisomes are light-harvesting antennae complexes which form regular arrays on the cytoplasmic face of the thylakoid membranes. These structures are primarily composed of the brilliantly colored, chromophore-bearing phycobiliproteins. Additionally phycobilisomes contain smaller amounts of proteins, termed linker polypeptides. The linker polypeptides determine the aggregation properties of the phycobiliproteins and are required for the assembly of phycobilisomes. The three-dimensional structures of two phycobiliproteins from the phycobilisomes of the cyanobacterium *Mastigocladus laminosus* have been determined through X-ray crystallographic analyses. All attempts thus far to obtain crystals from complexes of phycobiliproteins and linker polypeptides have failed. Because of the difficulties in obtaining crystals of phycobilisome subcomplexes containing linker proteins, we now are attempting to isolate pure "native" linker proteins. We hope to determine their structures either by crystallization and X-ray diffraction methods or by nuclear magnetic resonance (NMR) spectroscopy of the protein in solution.

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FOUR POLYPEPTIDES WITH CORE ANTENNA STRUCTURE IN *ECTOTHIORHODOSPIRA*

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The antenna complexes of purple non-sulphur bacteria are composed of oligomers consisting of α/β heterodimers. All species contain a core antenna complex which is closely connected to the reaction center. In some bacteria there are additional peripheral antenna complexes. Focussing on the primary structures one can find typical elements for either core antenna or peripheral antenna polypeptides. In a comparative sequence analysis we have investigated the polypeptides of the antenna complexes from the two sulphur bacteria *Ectothiorhodospira halochloris* and *Ectothiorhodospira halophila.* Besides one polypeptide, which seems to be a component of a peripheral antenna complex, two pairs of α/β-polypeptides exhibiting typical core antenna characteristics were found in both organisms. Of these two pairs however, one of them shows less homology to known antenna polypeptide sequences from purple bacteria than the other. These results could indicate a late evolutionary separation of the latter complex from the original core antenna structure.

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POLYMORPHISM OF CADMIUM-INDUCED MUSSEL METALLOTHIONEIN

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Metallothioneins (MTs) are ubiquitous sulphur-rich, heavy metal binding proteins whose biosynthesis is induced in response to heavy metal ions (Cd(II), Zn(II), Cu(I)), and other factors. Interest has grown in the nature of these proteins in the common sea mussel *Mytilus edulis* due to their possible utility as pollution indicator agents. Cadmium-induced MTs from *M. edulis* were shown to comprise two molecular mass classes of 10 and 20 kDa. The 10 kDa class was resolved by anion-exchange chromatography into four isoforms. Similarly, the 20 kDa class was resolved into three isoforms. Complete amino acid sequences of each of the seven isoMTs were determined. The three isoforms of the 20 kDa class were shown to possess linked peptides consisting of 71 amino acids. These peptides were distinct from the 72 amino acid peptides of the 10 kDa class. Isoforms from both classes exhibited homology to mammalian MTs. The significance of this homology is discussed.

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KINETICS OF METAL EXCHANGE IN METALLOTHIONEIN

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Metallothioneins (MTs) are suggested to be key compounds regulating the transfer of group-16 metals (Zn, Cd, Hg) within cells. We have now examined in a kinetic study at pH 8.6 the metal exchange between the homogeneously reconstituted rabbit liver isoforms MT-1 and MT-2. The velocity of exchange was measured by incubating for various time intervals radioactively labeled MT-1 (e.g., $^{109}Cd_7$-MT-1) with unlabeled MT-2 (e.g., Cd_7-MT-2) and subsequently separating the isoproteins by PAGE or ion exchange HPLC and counting their radioactivity. The results with the Cd- and Zn-derivatives show that 50% of the metal exchanges within minutes after mixing, although the metal-binding constants at pH 8.6 are known to be of the order of 10^{21} M^{-1} and 10^{17} M^{-1}, respectively. The exchange reaction rates were also found to be independent of the metal species and of protein concentration. Thus, we suggest, tentatively, that the rate-limiting step is an intramolecular isomerization of the metalloprotein into a form in which the ion is available for rapid exchange.

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ROLE OF LYSINE RESIDUES IN METALLOTHIONEIN (MT)

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The basic amino acid residues lysine and arginine are well conserved in the evolution of the MTs and have been suggested to contribute to the stabilization of the metal-thiolate clusters (Kojima *et al.*, Proc. Natl. Acad. Sci. USA 73, 3413 (1976); Pande *et al.*, Biochemistry 24, 6717 (1985)). We have now investigated this surmised role by subjecting the seven Lys of Cd_7-MT-2a and Zn_7-MT-2a from rabbit liver to carbamylation with KCNO or to guanidination with O-methylisourea. In both cases, all Lys were selectively modified, resulting in homogeneous derivatives as judged by compositional analysis, electrophoresis and gel filtration. Their UV absorption and circular dichroism features as well as the ^{113}Cd NMR spectra of derivatives substituted with this isotope were similar to those of the unmodified form. However, the loss of seven positive charges upon carbamylation resulted in an about fourfold decrease in the stability constants for Cd(II) and Zn(II), as measured by spectrophotometric pH titration and competitive chelation, respectively. By contrast, guanidination which preserves the positive charge had the effect of slightly increasing the stability of the metal-thiolate complexes. Thus, the basic residues are not essential to cluster formation. However, they contribute through their charge-balancing effect to metal cluster stability.

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MOLECULAR DYNAMICS SIMULATIONS OF METALLOTHIONEIN (MT)

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Based on the NMR-solution structure (Schultze, P. et al., *J. Mol. Biol.* (1988) **203**, 251) molecular dynamics were performed on Cd(II)-MT-2 from rat liver. The ten best NMR strucutres were refined by constrained and unconstrained energy minimization. The dynamics were calculated using the AMBER programme package (Weiner, S.J. et al. *J. Comp. Chem.* **1986**, 7, 230).
Analysis of the dynamic changes of the two Cd(II)-thiolate clusters showed that the fluctuations of the cadmium and sulfur atoms of both clusters are of similar magnitude as the deviations of the distance geometry strutures.
A combination of the structure of the two domains yielded to a hypothetical overall structure of Cd(II)-MT-2 which fulfills both the NMR constraints as well as the condition of energy relaxation.

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TETRAHEDRAL Hg(II)-TETRATHIOLATE PEPTIDE COMPLEXES AS MODELS FOR Hg_7-METALLOTHIONEIN (Hg_7-MT)

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In Hg_7-MT, Hg(II) is thought to be coordinated tetrahedrally to Cys in a Hg_3Cys_9 and a Hg_4Cys_{11} cluster (Bernhard et al., Inorg. Chim. Acta 79, 154 (1983)). As a model for analogous Hg(II) complexes, we have now synthesized the heterodetic cyclic dodecapeptide AcPCOCPOCOCRRV (O=ornithine). Addition of successive increments of Hg(II) to this peptide results in the formation of two distinct complexes with Hg-to-peptide stoichiometries differing by a ratio of 1:1.5. The first complex displays the features of mononuclear tetrahedral $HgCys_4$ coordination with an absorption shoulder at 280 nm, an associated negative natural CD band and a negative Faraday A-term MCD signal. The second complex manifests itself by a 20 nm red shift of both the absorption shoulder and the MCD signal, and the emergence of a multiphasic natural CD profile. It is a dimer formed from two mononuclear Hg-peptide complexes, linked through tetrahedral S-coordination by an additional equiv of Hg(II), thereby yielding a trinuclear Hg_3Cys_8 cluster. The spectroscopic changes associated with dimerization indicate transformation of four of the eight terminal thiolate ligands into bridging ligands and the generation of optical activity by excitonic coupling of thiolate chromophores within the cluster. Thus, these complexes are excellent models for Hg(II) binding in MT and related metalloproteins.

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SPECTROSCOPIC STUDIES ON CADMIUM SUBSTITUTED RUBREDOXIN

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The iron ion in native rubredoxin (Rd, *D. gigas*) has been replaced by cadmium *in vitro* to provide a model for the isolated metal binding sites in metallothionein (MT) at low metal occupancy. The binding of the $Cd^{(II)}$ ion to apo-Rd induces spectral features characteristic of cadmium thiolate complexes; in agreement with the ^{113}Cd NMR data. The difference (Cd-Rd vs. apo-Rd) electronic absorption spectrum reveals a band with a maximum at 245 nm (also seen in MT [1]) due to the charge transfer transitions (S→Cd) of the cysteine thiolates. Furthermore, the difference CD and MCD spectra of Cd-Rd support the evidence for the tetrahedral tetrathiolate binding of the cadmium ion into the binding site originally occupied by the iron ion. The presence of two bands in these spectra suggest that the difference absorption envelope contains at least two charge transfer transitions.
[1] H. Willner, M. Vasak and J. H. R. Kägi Biochemistry, 26, 6287 (1987).

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RAPID, EFFICIENT AND LOW-COST MANUAL ISOTHIOCYANATE DEGRADATION OF PROTEINS USING PVDF-TRANSFER MEMBRANES

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In the past decade improvements in protein sequencing methodologies were mainly aimed at obtaining N-terminal sequence information with approx. 50 to 500 pmole of material. Automatic or manual gas-phase sequencing methods using glass-filters certainly marked a breakthrough in this field. Very recently, polyvinylidene difluoride (PVDF) transfer membranes as protein carriers have been employed in a cross-flow reaction chamber of the newly designed Knauer (Berlin) sequencer. On this poster a manual method using PVDF membranes is described. It allows the analyses of many samples at a time and is suitable for use as a commonly available, low-cost method. In addition, PVDF membranes are most suitable in N-terminal sequence analyses of hydrophobic proteins or fragments (e.g. electroblotted samples from SDS-PAGE). PVDF-transfer membranes of 25 to 50 mm^2 are placed into standard insert glass tubes commonly employed in autoinjectors. The inserts are placed in standard glass holders equipped with teflon sealed caps. In this study protein fractions from gel-filtrations run in organic solvents as well as reversed-phase chromatographed polypeptides (0.1 to 0.5 nmole) have been applied in portions of max. 10 µl onto the PVDF-membrane. A detailed experimental protocol of the Edman degradation and the PTH-amino acid-HPLC chromatograms will be presented.

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ISOLATION AND CHARACTERIZATION OF THE ALLOPHYCOCYANIN COMPLEXES OF THE CYANOBACTERIUM *MASTIGOCLADUS LAMINOSUS*

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Cyanobacteria contain extramembraneous light-harvesting pigment-protein complexes, the phycobiliproteins. They are aggregated into large light-harvesting antennae, the phycobilisomes, which absorb light energy between 500 nm and 650 nm and transfer the exitation energy to the reaction center in the photosynthetic membrane. Allophycocyanin from *Mastigocladus laminosus* is a blue water-soluble pigment protein-complex and a component of the phycobilisome core. For crystallization trimeric allophycocyanin complexes $(\alpha^{AP}\beta^{AP})_3L_c^{8.9}$ were isolated and purified in preparative amounts by ion-exchange chromatography (Cellex D, Fractogel-DEAE and Mono-Q) and gelfiltration (BioRad P-60). Using the "Mini-Gel Electrophoresis-System" from BioRad we obtained high resolution of phycobiliprotein bands and we could distinguish four AP-bands instead of two. Recently a further microheterogenity was proposed for the α^{AP} and β^{AP}-chain. Although we isolated the same AP-complex (with the same spectroscopic data) the amino acid sequences of the observed four bands showed the known amino acid sequences of the pure α^{AP} and β^{AP}-chain in the first 20 degradation steps. We hope to obtain additional information concerning the core components by DNA-sequence analysis of AP-genes.

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ISOLATION AND CHARACTERIZATION OF DIFFERENT PHYCOCYANIN-LINKER POLYPEPTIDE COMPLEXES FROM THE CYANOBACTERIUM *MASTIGOCLADUS LAMINOSUS*

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Different phycocyanin-linker polypeptide complexes (containing colourless 34.5 kd, 31.5 kd, 29.5 kd linker polypeptides) and rod-core complexes were isolated by means of ion-exchange and gel-filtration chromatography from phycobilisomes of *Mastigocladus laminosus*. The size, composition, stoichiometry, spectroscopic properties of the complexes were determined. N-terminal amino-acid sequences of the three rod-core linker polypeptides (33 kd, 31.5 kd, 29.5 kd) are presented.

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N-ACETYLATION OF SEROTONIN USING ACETIC ANHYDRIDE

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N-acetylation of serotonin can be performed by two different methods, enzymatic or chemical using acetic anhydride. In order to find out the amount of serotonin which can be N-acetylated by the acetic anhydride method, solutions with a pH between 0 and 9.5 were tested on serotonin standards and on human aqueous humor. HPLC with electrochemical detection was the method of assay. Using the original method of Hammel et al (Anal Biochem 1978; 90: 840-843), which employs a pH=7.2, only 39.64 % of a sample of 20 ng/ml of serotonin were transformed into N-acetylserotonin. The best results were obtained with a pH=4.76 and 2% acetic anhydride. Under these conditions, 53.07% of the serotonin were N-acetylated. With samples of aqueous humor, only 14,74% of the natural serotonin was transformed. These results show that this method seems unable to N-acetylate all serotonin and that the pH is a critical factor in this process.

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ISOLATION OF RAT LIVER SPECTRIN AND IDENTIFICATION OF FUNCTIONAL DOMAINS.

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Spectrin associated with liver plasma membrane has been purified and its subunits isolated. The alpha-subunit retains the ability to bind both calmodulin and actin. The localization and structure of calmodulin-binding domains has been determined using fragments of the 240 kDa subunit obtained either by proteolytic or chemical digestion. A calmodulin-binding domain has been identified in the mid-region of the alpha-spectrin.

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MUTAGENESIS OF CARBOXYL-TERMINAL -CXXM MOTIF OF LAMIN PROTEINS REVEALS A CRITICAL ROLE IN ISOPRENYLATION AND NUCLEAR ENVELOPE ASSOCIATION

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The carboxy-terminus of nuclear lamins (-CXXM) resembles a C-terminal motif (-CAAX) of fungal mating factors and ras-related proteins that is subject to different types of post-translational modifications. These include proteolytic processing, isoprenylation and carboxyl methylation. By peptide mapping we show that both chicken lamins A and B_2 are processed proteolytically in vivo. However, whereas the entire -CXXM motif is cleaved from lamin A, at most three C-terminal amino acids are removed from lamin B_2. Following translation of cDNA-derived RNAs in reticulocyte lysates, lamin proteins specifically incorporate a derivative of ^{14}C-mevalonic acid (MVA), i.e. the precursor of a putative isoprenoid modification. Remarkably, no MVA is incorporated into lamin B_2 translated from a mutant cDNA encoding Ala instead of Cys in the -CXXM motif. Concomitantly, as demonstrated by analysis of transfected mouse cells, this mutated lamin B_2 fails to localize properly to the nuclear envelope, indicating that a -CXXM motif is important in envelope-association.

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PARTIAL STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF ANTIGENIC DETERMINANTS ON CYTOCHROME P-450IIB.

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15 monoclonal antibodies (mca) against the major phenobarbital inducible rat liver microsomal P-450 (P-450IIB) were tested for their ability a) to distinguish between different P-450 isozymes; b) to recognize V8-, chymotryptic- and tryptic peptides of P-450IIB; c) to inhibit the binding of cytochrome b_5; d) to inhibit P-450 catalyzed reactions; e) to inhibit the b_5-stimulation of P-450 catalyzed reactions.
The results can be summarized as follows: Four mca show an inhibition of P-450 catalyzed reactions. Two mca exhibit decreased b_5-binding spectra and in parallel also decreased b_5-stimulation of P-450 catalyzed reactions. Four mca seem to recognize sequential epitopes and are able to react with different peptides.

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TRAPPING OF TRUE ENZYME-SUBSTRATE INTERMEDIATES OF ASPARTATE AMINOTRANSFERASE

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Cryoenzymological tools were applied to render enzyme-substrate intermediates of aspartate aminotransferase (AspAT) accessible to kinetic, chemical and structural analysis. Starting with the pyridoxal form of AspAT, addition of the amino acids glutamate, aspartate or cysteine sulfinate at -60° C in the cryosolvent water/methanol resulted in the formation of stable intermediates absorbing at 360 and 430 nm. When mixing the pyridoxamine form of the enzyme with the keto acids 2-oxoglutarate or oxalacetate, respectively, another enzyme-substrate intermediate could be detected by a slight bathochrome shift (2 nm) of the pyridoxamine absorption band. The intermediates were further characterized by circular dichroism measurements at subzero temperature. The results indicate the trapped intermediates to be the external aldimine (i.e. the Schiff base between the pyridoxal form and the amino acid) and the ketimine (i.e. the Schiff base between the pyridoxamine form and the keto acid). Thus, these important intermediates of enzymic B_6 catalysis are now available for further chemical and cryocrystallographic studies.

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CHARACTERIZATION AND STRUCTURE OF TWO SUBUNITS OF THE RAT INTESTINAL ALCALINE PHOSPHATASE (IAP)

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The rat IAP has been purified to 90-95% homogeneity by a partly modified purification method reported for the calf IAP [Portmann & al., 1982, Helv. Chem. Acta, 65, 2688], introducing a chromatography on an affinity histidyl-phosphonic resin and a gel filtration on Superose 12 (FPLC). This resulted in the preparation of pure IAP (1870 U/mg, 25°C). The two subunits (L : light chain (62 kDa); H : heavy chain (87kDa)) of IAP have been isolated by PAGE-SDS with the help of prestained molecular weight standards (BioRad). The carbohydrates and the aminoacids composition of both subunits have been analysed. The aminoacid sequences of the L and H chains are reported :

L : V-I-P-V-E-E-E-N-P-V-F-H(?)-N-Q-Q-A-X-X-A-L-X-V-A-...;
H : V-I-P-V-E-E-E-N-P-A-F-X-N-Q-Q(?)-A-A-D-A-L-N-V-A-X-X-L-Q-P(?)-I-...

The IAP subunits show no sequence homology with the tissue-unspecific rat isoenzymes but some homology with IAPs isolated from other species.

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CHARACTERIZATION AND STRUCTURE OF THE RAT RENAL ALCALINE PHOSPHATASE (RAP)

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The rat RAP has been purified to 95% homogeneity by a partly modified purification method reported for the calf IAP [Portmann & al., 1982, Helv. Chem. Acta, 65, 2688]. The fractional ethanol precipitations, the salting-out and the CM-cellulose chromatography were replaced by an affinity chromatography (Sepharose hystidylphosphonic) and by a gel filtration (FPLC, Superose 12). This allowed to increase the yield resp. the purification degree to 11% resp. 3939 U/mg at 25°C (instead of 4% and 1300 U/mg with the former method). The RAP shows a single band on PAGE-SDS (72 kDa) and also one band on native PAGE (Coomassie Blue and specific staining). The sequence of the first 40 aminoacids of RAP is reported :

F-V-P-E-K-E-K-D-P-S-Y-W-R-Q-Q-A-Q-E-T-L-K-N-A-L-K-L-Q-K-L-N-T-N-V-A-W-N-I-I(or T)-M-F-...

This sequence is nearly 100% homologous to that of the tissue-unspecific rat isoenzymes. The aminoacids composition and the carbohydrates content have also been determined.

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ON THE STRUCTURE OF CALF INTESTINAL ALKALINE PHOSPHATASE

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By screening a calf duodenum cDNA library we have isolated a clone encoding the calf intestinal alkaline phosphatase (CIP), merely lacking the 5' end. The 2167 bp long insert codes for a 433 amino acid polypeptide which is missing the aminoterminus. This translation product shows 76% identity to the human intestinal enzyme (HIP). A highly hydrophobic sequence at the carboxyterminus probably represents a transient membrane-anchoring peptide, being replaced by a phosphatidylinositol-glycan moiety. Four potential N-linked glycosylation signals are found, three of which are conserved in HIP. So far, two sugar chains have been assigned to positions 249 and 410 which represent the two last glycosylation sites.

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MOLECULAR ANALYSIS OF HEPATIC GAMMA-GLUTAMYL TRANSPEPTIDASE

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Activity of gamma - glutamyl transpeptidase (GGT) is known to be essentially absent in normal rat hepatocytes, as opposed to intrahepatic bile ductular epithelial cells. Accordingly, we were unable to detect in normal hepatocytes GGT messenger RNA, and also the constitutive 58/27 kD dimeric protein described in tissues with physiological GGT expression was virtually absent. However, using Western blot techniques we found that normal hepatocytes contained higher molecular weight forms of the enzyme (65, 74 and 83 kD) that were not expressed in bile ductular epithelial cells. Induction of GGT activity in hepatocytes was accompanied by a simultaneous increase of the active 58/29 kD dimeric form of the enzyme, which was preceded by appearance of messenger RNA and induction of the high molecular weight proteins. In vitro turnover studies suggest that the high molecular weight forms represent precursor proteins that are not processed under physiological conditions in hepatocytes. Supported by the SANDOZ-Stiftung.

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CHARACTERIZATION OF THE HUMAN NEUTROPHIL NADPH OXIDASE BY ISOELECTRIC FOCUSING ELECTROPHORESIS

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To further analyse the different components of the membrane bound NADPH oxidase from human neutrophils, nitroblue tetrazolium and tetramethylbenzidine-H_2O_2 staining methods were used to simultaneously visualize the $O_2^{\cdot-}$-forming activity and cytochrome b on isoelectric focusing electrophoresis (IEF) gels (pH gradient 3,5-9,5). Heme proteins were found to migrate to pIs 8,1 and 10,4 while proteins staining for NBT reduction migrated to pIs 5,0-5,5 and 6,0-6,3. The IEF gels were sliced, then run on SDS-PAGE electrophoresis and silver stained. The NBT stained proteins (pI 4,9-5,3) showed prominent bands at 66,60,20 kDa, consistent with the presence of the NADPH binding protein (66 kDa), while proteins focusing at pI 5,9-6,2 showed prominent bands at 70 and 43 kDa. The resolution of the components of the active oxidase complex does not affect the production of superoxide, indicating that cytochrome b does not participate in electron transfer at least in vitro.

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REDUCTION OF SUCCINIC SEMIALDEHYDE BY SUCCINIC SEMIALDEHYDE REDUCTASE AND ALDEHYDE REDUCTASE FROM HUMAN BRAIN

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The neuromodulator gamma-hydroxybutyrate (GHB) is synthesized in vivo from GABA via the intermediate succinic semialdehyde (SSA). Two enzymes, SSA reductase and aldehyde reductase (EC 1.1.1.2), catalyze the NADPH-dependent reduction of SSA to GHB in human brain. We here report the results of a comparative kinetic study of the two enzymes. At pH 7.0 the Km values of SSA reductase for NADPH and SSA were 5 and 12 µM, those of aldehyde reductase 2 and 20 µM, respectively. With both enzymes product inhibition by NADP was competitive relative to NADPH and non-competitive relative to SSA. GHB was a noncompetitive inhibitor relative to both substrates.The results suggest that the two enzymes follow an ordered sequential mechanism with NADPH binding to the enzyme first.

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CYCLOSPORIN A SLOWS COLLAGEN TRIPLE HELIX FORMATION - INDIRECT EVIDENCE FOR A PHYSIOLOGIC ROLE OF PEPTIDYL-PROLYL CIS-TRANS ISOMERASE

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Peptidyl-prolyl cis-trans isomerase (PPIase) accelerates rate-limiting cis to trans isomerization steps during folding of proteins in vitro, but is not yet securely identified with any specific physiologic role. PPIase and the cyclosporin A (CsA) binding protein cyclophilin are identical, and PPIase activity is inhibited by CsA. We investigated the influence of CsA on the in vivo folding of procollagen I, which is limited in rate by cis-trans isomerization steps. In chick embryo tendon fibroblasts, CsA slows the folding of procollagen I: the time needed for 50% of the molecules to reach complete helical conformation was 13.5 min in the presence, and 8.5 min in the absence of 5 µM CsA; and the products, k x K, of the rate constant (k) and the equilibrium constant (K), were 1,30 and 2,10 s^{-1}, resp.. In cultured human fibroblasts, CsA caused posttranslational overmodification and increased intracellular degradation, and hence decreased production, of collagens I and III. These observations provide the first, though indirect evidence for a physiologic role of PPIase.

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AUTOLYSIS OF CALPAIN-LIKE PROTEASE FROM *ALLOMYCES ARBUSCULA*

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The calpain-like protease from *Allomyces arbuscula* shares many functional properties with vertebrate calpains but is significantly different in its structural organization (Ojha and Wallace, 1988; Ojha, 1989). The native *Allomyces* enzyme has a relative molecular mass of 84-90 kDa. In an earlier SDS-PAGE analysis of fractions eluting from the Phenyl-sepharose affinity column with EGTA as eluent we found a single band of 40 kDa by silver nitrate and coomassie staining although activity gel of certain fractions eluting earlier than the peak showed a minor 43 kDa band (Ojha and Wallace, 1988). In a rapid purification procedure devised to prepare enzyme with native phosphorylation -state intact using 50 mM NaF as alkaline phosphatase inhibitor (we also found that NaF is a potent inhibitor of *Allomyces* calpain-like enzyme), we could show the presence of 43 kDa and 40 kDa peptides in equal amounts, both containing phosphorylated serine residues (Favre and Ojha, 1990). We now show that an intermediate, a minor 42 and major 40 kDa peptides are derived from the 43 kDa peptide through autolytic processing. We also show that further autolysis leads to the accumulation of a 31 kDa peptide which whilst retaining its Ca^{2+}-binding fragment, becomes proteolytically inactive.

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PHARMACOKINETICS OF THE RECOMBINANT ALPHA-1-ANTITRYPSIN (rAAT) IN THE RAT

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Recombinant α_1-antitrypsin (rAAT) is a potent inhibitor of neutrophil elastase (NE) and may therefore be of therapeutic value in diseases such as lung emphysema and cystic fibrosis. Uncontrolled degradation of connective tissue by NE is believed to play a major role in the development of these diseases. Pharmacokinetics of rAAT was studied after i.v. and intratracheal application to rats. Levels of rAAT were measured in plasma and lung lavage by an ELISA procedure. The half life of the distribution and elimination phase after i.v. bolus injection of rAAT was determined to be 27 and 100 minutes, respectively. Levels of rAAT in lung lavages after intratracheal administration declined with a half life of approximately 12 hours. Plasma levels of rAAT peaked 4-8 hours after intratracheal application indicating that rAAT transferred through the lung interstitium into the blood stream. These results show that rAAT is rapidly eliminated from the blood after i.v. injection. However, after intratracheal application, rAAT remains in the lung for an extended period of time. This indicates that in patients with lung emphysema or cystic fibrosis, intrapulmonal application of rAAT may provide adequate protection against neutrophil elastase.

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HIGHER MOLECULAR WEIGHT FORMS OF BASIC FIBROBLAST GROWTH FACTOR (bFGF) IN BRAIN TISSUE

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Basic fibroblast growth factor (bFGF) was described originally to be an 18 kD polypeptide initiated by a methionine. Recently N-terminal extended forms of bFGF have been identified which use an alternative start codon, CUG rather than AUG, for initiation of translation. By Western blot we analysed mouse, rat and bovine brain for bFGF. The predominant form in these brains was the 18 kD bFGF. In the rodent brains higher molecular weight forms were found as doublets of about 22 kD, whereas in bovine brain (as in human) higher forms were found at 21 kD and 22.5 kD. There is a 98 % amino acid homology for the rat and human 18 kD bFGF, but the N-terminal extensions predicted from the cDNAs have only 32 % amino acid homology. In both species, however, the N-terminal extension is very cationic, is high in glycine, arginine, and alanine, and includes the motif GGRGRGRG in which the arginines may be methylated.

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PREGNANCY-ASSOCIATED PLASMA PROTEIN B (PAPP-B): ABOUT ITS MOLECULAR WEIGHT, ITS STRUCTURE, ITS PHYSICO-CHEMICAL NATURE

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The Pregnancy-Associated Plasma Proteins, PAPP's, were described in the early seventies by T.M.Lin [1]. He described a family of four large glycoproteins using immunological methods. One of them, the PAPP-D, was soon recognized to be immunologically identical to the HPL; another, the PAPP-C, revealed itself as identical to the SP_1, and the PAPP-A is presently known to have structural similarities with the α_2M. The PAPP-B, however, remained unstudied until recently [2]. Its molecular weight seems to be definitively established as 1,100 kD. PAPP-B splits into two identical subunits of 530 kD when treated with SDS, and into four smaller components of 75.3, 63.8, 52.4, 20.1 kD upon reduction with β-mercapto-ethanol. The molar ratio determined by gel scanning is 4:2:1:2. On western blots the preparation did not show any contaminations with albumin, α_2M, IgM, IgA, IgG. The sugar content is 8.3, 14.7, 14.8, 0.0 % per subunit. The titration curve and the western blot of IEF showed a pI of 5.3 and 5.27 respectively. The molar coefficient of extinction at 280 nm is 6.5×10^9 and the Trp/Tyr ratio is 1.1.

References:

[1]T.M.Lin et al. Am.J.Obstet.Gynecol. (1974), 118, 223-234.
[2]M.Bossi et al. FEBS Meeting 89 (1989), Abstracts, TH 061.

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SELECTIVE BINDING OF HISTONES TO POLY(ADP-RIBOSE)

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Transient modification of certain DNA-binding proteins by and including poly(ADP-ribose)-polymerase increases in the presence of DNA single- and double-stranded breaks. Poly(ADP-ribose) [poly(ADPR)] thus formed may function to temporarily displace proteins (ie. histones) from DNA in order to allow access of repair/replication enzymes. We have shown, using mobility-shift gels, that DNA is released from core particles upon poly(ADPR) formation. Further analyses revealed that histone binding to free poly(ADPR) rendered the polymer phenol-extractable in a highly selective manner. 1) Histones bound preferentially to the longer, more complex polymers. 2) The effectiveness of polymer extraction by equimolar amounts of histone was in the order H1>H2A>H2B=H3>H4. 3) Histone binding protected the polymer from digestion by venom phosphodiesterase. Furthermore, the above interactions could be eliminated in the presence of SDS or proteinase K. These observations support a model in which poly(ADPR) shuttles proteins off and on DNA.

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OmpA GENE DISRUPTION TO SIMPLFY ISOLATION OF E-COLI PORIN

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Isolation of native K12-OmpF porin from BE cells was impaired by OmpA contamination. This initiated our attempts to disrupt the chromosomal OmpA gene. Using insertional mutagenesis on a plasmid carrying OmpA, streptomycin (Sm) and spectinomycin (Spc) resistencies were cloned into a BamH1 site of this gene. This plasmid was linearized and transformed into the porin⁻ E.coli BZB1107. Sm/Spc resistant colonies were then grown in presence of K3, a phage using the OmpA protein as receptor. Surviving cells were analyzed for their OmpA content by a Western blot and revealed no detectable amounts of OmpA. Subsequent transformation with a plasmid carrying the K12-OmpF gene allows the extraction and isolation of highly purified native porin trimers in a few steps.

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REGULAR ARRAYS OF THE PHOTOSYSTEM I REACTION CENTRE

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We have reconstituted two-dimensional (2D) ordered arrays of the photosystem I reaction centre from mixed detergent-lipid and detergent-protein micelles. A special open dialysis chamber was used that allows the lattice quality to be monitored by electron microscopy during dialysis. Preliminary reconstitution experiments of the trimeric PS I rc complex with DMPC yielded tightly packed membranes of PS I rc monomers that did not exhibit crystallinity but showed an absorption spectrum characteristic for the native PS I rc complex as well as full enzymatic activity. We then found conditions to grow two crystal forms with unit cell dimensions: (i) a=b=8.5 nm, 60° and (ii) a=b=15.4 nm, 60°. Negatively stained 2D crystals are now analyzed by electron microscopy and image processing to determine the 3D structure of the PS I rc monomer at low resolution.

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A S. CEREVISIAE PROTEIN ATTACHED TO THE MEMBRANE BY A GLYCOSYL-PHOSPHATIDYLINOSITOL-ANCHOR: CLONING OF THE GENE AND ANALYSIS OF THE SIGNAL FOR ANCHOR ATTACHMENT

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We have cloned and sequenced the gene encoding a 125 kD glycoprotein of unknown function that has been previously shown to be attached to the membrane by a glycosyl-phosphatidylinositol (GPI)-anchor (1). The sequence codes for a polypeptide (559 amino acids, predicted molecular weight: 59.5kD) with a cleavable NH_2-terminal signal sequence, 10 potential N-glycosylation sites, potential O-glycosylation sites and a hydrophobic COOH-terminal peptide that is suspected to function as a signal for the attachment of a GPI-anchor. To prove this hypothesis we are investigating the effects of COOH-terminal deletions on anchor attachment and we have constructed COOH-terminal fusions of the potential signal peptide to proteins that are normally secreted (invertase and the BAR1 protease). Eventually, these constructions may enable us to screen for mutants in the mechanism of anchor attachment.

1) Conzelmann et al., (1988), EMBO J., 7, 2233-2240

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COMPLETE STRUCTURE OF THE GLYCOSYL-PHOSPHATIDYLINOSITOL MEMBRANE ANCHOR OF *LEISHMANIA* SURFACE PROTEASE

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The protozoan parasite *Leishmania* expresses at its surface a membrane-bound metalloprotease of approximatively 63 kDa.This protease is anchored in the plasma membrane by a glycosyl-inositol phospholipid.

The lipidated anchor was prepared and its structure was elucidated. The four amino acids found at the C-terminus of the mature protease are located 25 amino acids before the predicted C-terminus of the precursor protein. The lipid moiety contains 1-O-alkyl,2-O-acyl glycerol. The carbohydrate portion is formed by ethanolamine PO_4-Man(α1-2) Man(α1-6)Man(α1-4)GlcN(α)*myo*-inositol. This carbohydrate structure is similar to those found in the anchors of *Trypanosoma* VSG and rat brain Thy-1 but does not contain phosphoethanolamine or carbohydrate side chains.

It is possibly the simplest structure that will be found in this type of membrane anchor.

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STRUCTURAL ANALYSIS OF GLYCOPHOSPHATIDYLINOSITOL ANCHORS OF SACCHAROMYCES CEREVISIAE.

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Saccharomyces cerevisiae contains numerous membrane glycoproteins which are anchored in the lipid bilayer via a glycophosphatidylinositol moiety. To elucidate the structure of this glycolipid anchor we prepared micromolar quantities of this compound from a crude membrane fraction. The carbohydrate moiety of this anchor contains ethanolamine and glucosamine in equimolar amounts. Nitrous acid degradation, treatment with hydrofluoric acid and enzymatic treatments of the thus generated fragments indicate that the core oligosaccharide which links the protein to the lipid moiety is similar in yeast and in mammalian organisms. In contrast the lipid moiety of yeast is significantly different from mammalian organisms in that it is resistant to mild alcaline methanolysis and consists most likely of a ceramide.

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COMPARATIVE STUDIES ON THE PRIMARY STRUCTURE OF ACETYLCHOLINESTERASE FROM BOVINE CAUDATE NUCLEUS AND BOVINE ERYTHROCYTES

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Comparison of the amino acid sequence of G_2-acetylcholinesterase (AChE) from bovine erythrocytes and G_4-AChE from bovine caudate nucleus revealed a high homology between the two enyzmes. The first 29 residues of the N-terminal sequence were identical. In addition the amino acid sequences of 4 peptides generated by tryptic and cyanogen bromide cleavage were identical for bovine erythrocyte and brain AChE. Comparison of these sequences to that of fetal bovine serum AChE showed differences in residue 16, 181, 212 and 216. These divergences suggest that fetal and adult forms of AChE are encoded by different genes and are not generated by different splicing events. G_4-AChE from bovine brain is membrane bound through a non-catalytic subunit of 20 kDa linked to two of the four catalytic subunits by disulfide bridges. Using [^{125}I]TID-labelled AChE we could show that there is at least one cleavage site for trypsin in the membrane anchor. Furthermore our results indicate that the hydrophobic domain contains a fatty acid which is covalently linked to a cysteine residue within the peptidic part of the membrane anchor.

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ANTIBODIES AGAINST THE CROSS REACTING DETERMINANT OF GPI-ANCHORED ACETYLCHOLINESTERASE

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Dimeric acetylcholinesterase (AChE) is membrane bound by a glycosyl-phosphatidylinositol (GPI) anchor covalently attached to the C-terminus of the protein. The complex glycan moiety contains an antigenic epitope which is only revealed after removal of the diradylglycerol by phosphatidylinositol-specific phospholipase C (PI-PLC) but is cryptic in the native amphiphilic form. This epitope is called cross reacting determinant (CRD), since it is present in most GPI bearing proteins after PI-PLC digestion. Polyclonal antibodies were raised against the CRD of AChE. The anti-CRD-antibodies recognized only the PI-PLC treated hydrophilic forms of bovine erythrocyte and Torpedo AChE and of variant surface glycoproteins from trypanosomes but not the corresponding amphiphilic proteins. Competition experiments showed that myo-inositol 1,2-cyclic phosphate and glucosamine inhibited the binding of the antibodies to the CRD. Reductive methylation of glucoseamine in the anchor also led to markedly reduced binding of the anti-CRD-antibodies. The amphiphilic methylated enzyme is less sensitive to digestion with PI-PLC than the non-methylated form. From these results we conclude that myo-inositol 1,2-cyclic phosphate and glucosamine, especially the free amine group of this residue, contribute significantly to the epitope recognized by our anti-CRD-antibodies.

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IDENTIFICATION OF A GLYCOSYL-PHOSPHATIDYL-INOSITOL SPECIFIC PHOSPHOLIPASE D (GPI-PLD) IN BOVINE BRAIN

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In recent years, an increasing number of proteins were shown to be lipid anchored by a covalently attached GPI residue. In mammalian cells, little is known about GPI-specific phospholipases (GPI-PL) which might play a role in the metabolism of GPI-anchored proteins. In order to identify GPI-PL, a rapid and sensitive assay for such enzymes was developed using acetylcholinesterase from red cells as substrate. With this assay, we established the presence of a GPI-PL in bovine brain. GPI-PL is soluble and could be partially purified by a heat step followed by chromatography on DEAE cellulose and Sepharose CL-6B. GPI-PL had a high affinity for the anchor of the substrate (K_m = 52 nM), and it did not degrade PC and PI. Chemical modification of the substrate caused a marked decrease in the cleavage rate. Since GPI-PL released phosphatidic acid from the substrate, the cleavage specificity was that of a phospholipase D. GPI-PLD was inhibited by an organo-mercurial o-phenantroline and EGTA, and stimulated by Ca^{2+} in µM concentrations. As suggested for GPI-anchored proteins, GPI-PLD from brain might be of importance in brain development.

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CHANGES IN LIPID COPMPOSITION OF THE PLASMA MEMBRANE ASSOCIATED WITH SEMLIKI FOREST VIRUS INDUCED CELL-CELL FUSION

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Membrane fusion implies mixing of two lipid bilayers followed by a reorganization of the bilayer structure. Evidence from several laboratories emerged that changes in lipid composition during this process might occur or even play a crucial role in the fusion process. Using Semliki Forest virus induced cell-cell fusion at low pH as a model we characterized the lipid composition of the plasma membrane at various times during polykaryon formation. Clear changes correlating with the fusion process were observed in PE, diacylglycerol and fatty acid contents of the plasma membrane. A possible role of these lipid alterations for the fusion will be discussed.

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KARYOPHILIC PROPERTIES OF SEMLIKI FOREST VIRUS (SFV) NUCLEOCAPSID (C) PROTEIN

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We have shown previously that SFV C-protein molecules, delivered by electroporation or by liposomes, partition rapidly from the cytosolic compartment into the nucleus were they accumulate in the nucleolus (Michel et al., manuscript submitted). Here we show that nuclear transport of delivered C-protein molecules can be reversibly arrested by metabolic inhibitors, demonstrating that nuclear trafficking of C protein is energy dependent. Furthermore, the lectin wheat germ agglutinin prevents transfer of C protein through nuclear pores. This finding substantiates our observation that nucleoli isolated from SFV infected cells at different times post infection contain increasing amounts of newly-synthesized C protein. Taken together, these observations support the hypothesis that the C-protein molecules contain a nuclear target sequence(s).

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TEMPERATURE DEPENDENCE OF SEMLIKI FOREST VIRUS (SFV) *PRODUCTION IN AEDES ALBOPICTUS C6/36 CELLS*

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C6/36 cells grow at 28°C. These cells exibit two pathways of viral maturation, namely intracellular (IC) and plasma membrane budding. In this study we have investigated, whether the low temperature at which C6/36 cells grow, is responsible for IC maturation. Our data reveal that the shedding of infectious virus was strictly temperature dependent. Decreasing the temperatures led to a concomitant reduction in virus titers. At lower temperatures longer incubation times were needed to yield the highest titers. Below 14°C viral shedding remained at background levels. However, uridine and methionine incorporation occurred even at 8°C indicating that the biosynthesis of macromolecules was still functioning. We are investigating now whether the block detected may either be due to an inhibition of IC transport of viral envelope proteins or to an inhibition of budding itself.

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THE ACYLATION OF SEMLIKI FOREST VIRUS (SFV) GLYCOPROTEINS IN INFECTED AEDES ALBOPICTUS C6/36 CELLS

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In C6/36 cells, in contrast to mammalian cells, intracellular alphavirus budding occurs and the cleavage of p62, the precursor of the envelope proteins E_3 and E_2, starts early after biosynthesis. The relation of virus maturation to complex posttranslational modifications such as endoproteolytic cleavage of precursors and acylation is unclear. Therefore we investigated where and when the SFV glycoproteins were acylated. Our results show that (i) the precursor p62 and the envelope proteins E_1 and E_2 are acylated, (ii) the linkage of the fatty acids to the proteins is a hydroxylamine-labile thioester, (iii) all newly acylated viral proteins are sensitive towards Endo-H digestion. This indicates that acylation is an early phenomenon in C6/36 cells. On the other hand, we found that the viral proteins were continuously labeled with [^{3}H]palmitic acid, even 2h after inhibition of protein synthesis with cycloheximide. This suggests that a late acylation of the viral proteins may also occur.

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THE EFFECT OF PROCESSING INHIBITORS ON THE FORMATION OF N-LINKED OLIGOSACCHARIDES IN SEMLIKI FOREST VIRUS-INFECTED **Aedes albopictus** C6/36 CELLS

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We have investigated the processing of Asn-linked oligosaccharides of Semliki Forest virus in C6/36 cells. Aedes albopictus cells were labeled with [^{3}H]mannose or [^{35}S]methionine for 20-24 h in media containing 1-deoxynojirimycin, 1-deoxymannojirimycin, or swainsonine. Analysis of the oligosaccharides obtained after Endo-H or Endo-F digestions showed that in the presence of trimming inhibitors, only high mannose structures were detected. The most impressive effect was found with 1-deoxymannojirimycin. The virions produced carried a homogeneous glycan of the form $Man_8GlcNAc_2$. However, these virions were fully competent. Without the inhibitors, the oligosaccharides were extensively processed. Their structures were identical to the trimannosyl-core of vertebrate cells. In addition, a modified oligosaccharide structure consisting of the trimannosyl "core" with a terminal GlcNAc was found.

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SFV SPIKE PROTEINS FUNCTION AS ION (H^+) CHANNELS

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Exposure of Semliki Forest virus (SFV)-infected Aedes albopictus cells to mildly acidic pH (< 6.2) results in a proton influx into the cell which correlates with cell-cell fusion. So far it was unknown whether viral or cellular membrane proteins are responsible for this proton influx. We showed that viral envelope proteins act as proton channels as demonstrated on intact and protease digested SFV virions. The nucleocapsid's sedimentation behaviour which is known to be different for isolated capsids after neutral (150+/-2 S) or acid (166+/-3 S) treatment was used to show this effect. Nucleocapsids of acidly treated intact virions had an S-value of 162+/-1 in contrast to neutraly treated intact virions with a capsid of 153+/-2 S. As a control protease digested virions were acidly treated. They contained a capsid of 154+/-2 S. These results demonstrate that intact envelope proteins are required for proton translocation.

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CHEMICALLY INDUCED PLASMA MEMBRANE VESICLES CAN BE USED AS A TOOL TO INVESTIGATE THE BINDING MECHANISM OF SEMLIKI FOREST VIRUS (SFV)

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SFV enters a cell via the endocytotic pathway. The initial binding of the virus to the plasma membrane is necessary for viral uptake into the cell. In this study we set out to find a method which would enable us to investigate the viral binding per se. We have therefore tried two different approaches. (i) By using lower temperatures, it was possible to reduce the rate of endocytosis of SFV in Aedes albopictus cells. However, we were not able to block this process at a convenient temperature at which the biochemistry of binding could be studied. (ii) In a second approach we have used chemically induced plasma membrane vesicles. These vesicles can be obtained from Vero cells and a number of other cell types by treatment with low concentrations of formaldehyde and dithiothreitol. Evidence is presented showing that SFV binds to these vesicles. Since no endocytosis occurs in these vesicles, we consider them to be an useful tool to study the adsorption mechanism.

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TUMOR CELL GROWTH INHIBITION BY A LIPOSOME ENTRAPPED IRON CHELATOR AND PROTEINASE INHIBITOR

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The aromatic poly-amidine TAPP-Br (the bromo derivative of 1,3-di-(p-amidinophenoxy)-2,2-bis-(p-amidinophenoxymethyl) propane) and the iron chelator desferrioxamine - both highly water-soluble compounds - are known to inhibit the growth of tumor cells in vitro. We have shown that this anti-tumor activity in both cases is increased when the drugs are entrapped into egg phosphatidyl choline multilamellar vesicles. The antiproliferative activity of the free and liposome entrapped drug has been tested by using different tumor cell lines, including human breast, kidney carcinomas and melanoma and murine erythroleukemic Friend cells. In all cases tested, the entrapped compounds were found to be more efficient than the free drugs: 2 to 6 times lower amounts are required in both cases to obtain a 50% cell growth inhibition when compared with the free drug.

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SOLUBILIZATION OF BACTERIAL CELLS IN ORGANIC SOLVENTS AND PETROL

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Bacterial cells and cell organelles can be solubilized into organic solvents by use of reverse micellar systems (microemulsions), i.e. a ternary system consisting of an organic solvent (e.g. isooctane, natural and mineral oil, petrol), a surfactant (e.g. AOT, Tween, phospholipids) and a small amount of water (1 to 5% v/v). The solubilization of Yeast, E. coli, Pseudomonas sp., Bacillus subtilis, and Cyanobacteria has been investigated. Clear, thermodynamic stable apolar solutions, consisting of more than 90% of the org. solvent, and containing up to several millions cells per ml, can be obtained. Yeast cells are able to maintain their viability up to several weeks, and cell strains having been previously in the org. media give better stability and activity response after resolubilization. Particularly interesting is the solubilization and the maintenance of viability of bacterial cells in mineral oil and its distillation products, like naphta or gasoline. In fact, this could lead to microbiological applications.

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ON THE STRUCTURE OF MICROEMULSION LECITHIN GELS

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Solutions of lecithin in an organic solvent can be transformed into a gel-like, viscoelastic solution by the addition of a small amount of water. Dynamic light scattering and small-angle neutron scattering investigations indicate the presence of a transient network formed by entangled cylindrical reverse micelles. The results were compared with recent theoretical and experimental work on the dynamic properties of semi-dilute polymer solutions and viscoelastic ionic micellar solutions.

These novel gel-like materials are not only interesting because of their structural and dynamic properties, but also in view of their capability of solubilizing and immobilizing bioactive guest molecules such as enzymes as drugs. This makes them potentially useful for a variety of biotechnological, chemical, pharmaceutical and medical applications.

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LIPID BEHAVIOR IN RED BLOOD CELL VESICULATION

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Erythrocytes release membrane vesicles under various conditions. Prior to vesicle release, a transformation of the original discocyte to an echinocytic cell shape can be observed, and the final step is the fusion of membrane domains at the tips of the echinocytic protrusions of the cell. The vesiculation process can thus be used as model system in studies on the mechanism of membrane fusion.
In the present study, vesicle release was obtained either by ATP-depletion, loading of the cells with Ca^{++}, or incubation with dimyristoylphosphatidylcholine (DMPC). The released vesicles were isolated by low speed centrifugation and, to remove lysed red cells and DMPC contaminations, were purified by gel filtration on a Sephacryl S-1000 superfine column, followed by Percoll density gradient centrifugation. Membrane phospholipid fatty acid composition was examined by GLC. In all preparations the quantitative ratio of endogenous phospholipids and fatty acids was the same in red cells, vesicles and vesiculated cells. The results indicate that the fusion event involved in vesicle release does not require a major rearrangement of membrane phospholipids.

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A NEW PHOSPHOLIPID ANALOGUE FOR PHOTOCOUPLING OF SOLUBLE PROTEINS TO LIPOSOMES

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Photoactivable lipids N'-(1,2-diacyl-sn-glycero-3-phosphatidyl-ethyl)-N-(m-3-trifluoromethyl-diazirine)-phenyl))-thiourea (PED) have been applied for light-induced binding of soluble proteins to the outer surface of liposomes. PED-lipids combine the advantages of facile synthesis and timed carbene reactivity by photoactivation at wavelengths which are non-destructive to proteins. C_{14}-, C_{16}- and C_{18}-analogues have been prepared from synthetic phosphatidylethanolamines and 3-(trifluoro-methyl)-3-(m-isothiocyanophenyl)diazirine. Transition temperatures for C_{14}- and C_{16}-analogues were $10^{o}C$ and $28^{o}C$ respectively. Photolabeled lipids were recognized as substrates and cleaved by phospholipase A_2 from Naja naja. C_{14}-PED formed liposomes in aqueos media. PED has been incorporated into liposomal membranes with varying amounts of non-photoactivable lipids. Formation of an inner aqueous space in PED liposomes has been ascertained by trypsin entrappment. Light-dependent immobilization of proteins ocurred upon photoactivation (356 nm) of PED-containing liposomes in presence of soluble proteins. Therewith a promising new method for the formation of proteoliposomes is established.

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RAPID ANALYTICAL AND PREPARATIVE ISOLATION OF INTACT ZYMOGEN GRANULES BY FREE FLOW ELECTRO-PHORESIS.

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Zymogen granules are organelles of the pancreatic acinar cell which store secretory proteins preparatory to secretagogue induced secretion. In order to obtain an understanding of the zymogen granule membrane structure and composition it is important to have highly purified intact zymogen granules. The procedure which we use, permits analytical and preparative isolation of intact zymogen granules. The procedure relies on a combination of density gradient centrifugation and free flow electrophoresis. It yields a fraction of highly purified intact organelles as measured by OD at 280 nm and amylase and 5'-nucleotidase activity (zymogen granule marker enzymes). Two distinct peaks of amylase were detected, one of which also contained 5'-nucleotidase activity. The activity of 5'-nucleotidase in a fraction which also contain amylase activity probably reflects the presence of intact zymogen granules.

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ROLE OF THE INTRACELLULAR pH ON ADRENAL MEDULLARY SECRETION.

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Left adrenal glands of male rats were perfused in vitro with a Ca^{++} free Krebs-bicarbonate sol. at 0.2 ml/min. at $36^{+1}{}^{o}C$. The gland was stimulated during 30 sec. by a CO_2 enriched solution (equilibrated during 5,10,20 and 30 sec) at 15 min. interwals. Puls acidification of the cytoplasma with a CO_2 enriched sol. preferentially stimulated adrenaline (adr) secretion in relation to the equilibration time with CO_2. The PhospholipaseC inhibitor neomycine ($10^{-3}M$) or the calmoduline antagonist trifluoperazine($10^{-5}M$) significantly depressed secretion induced by intracellular acidification. TMB-8 ($10^{-5}M$) an antagonist of the intracellular Ca^{++}, completely blocked acidification-induced adr secretion. Caffeine which mobilizes Ca^{++} from intracellular stores, increased the secretion of both catecholamines adr. and noradrenaline.

Results indicates that, adrenaline containing cells or vesicals may behave as an acide sensor, and also sugest that the Ca^{++} thersholds of two types of vesicals for secretion are different.

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INHIBITION OF CHROMAFFIN CELL EXOCYTOSIS BY AN ANTI-MEMBRANE PROTEIN ANTIBODY IS REVEALED BY CAPACITANCE MEASUREMENTS

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The tight temporal and spatial regulation of exocytosis could be mediated by specific recognition molecules on the secretory vesicle and the plasma membrane respectively. Such intracellular, membrane-bound ligands and receptors could hold the secretory vesicles in a position ready to fuse instantaneously upno stimulation. One possible component of such a recognition site on the plasma membrane is a 51kDa protein that has been isolated from bovine adrenal chromaffin cells. This protein binds selectively to chromaffin granules, the secretory vesicles of these cells. Using the sensitive cell membrane-capacitance measurement we show that antibodies against this membrane-anchored, chromaffin granule-binding protein completely block the fast phase of exocytosis. We thus demonstrate the functional involvement in exocytosis of a plasma membrane protein with a high affinity for secretory vesicles.

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SECRETION OF AN Mr 60'000 GLYCOPROTEIN BY BENOMYL-TREATED CELLS OF NEUROSPORA CRASSA.

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In the presence of the microtubule inhibitor benomyl at micromolar concentrations, cells of *Neurospora crassa* wild type strain St. Lawrence 74A were found to secrete high amounts of an Mr 60'000 protein into the culture medium (about 35 µg/ml after a 12 h treatment). The secretion also occurred after treatment with the other antitubulin drugs carbendazim (MBC), nocodazole, thiabendazole, griseofulvin, vincristin and vinblastin. This secretion is apparently induced by the specific action of benomyl on *N. crassa* ß-tubulin as no secretion of the Mr 60'000 protein could be detected after treatment of the benomyl-resistant mutant bml 511 (r), mutated in its ß-tubulin gene (Orbach et al. 1986). The secretion was abolished by 12 µM cycloheximide and by 5µM monensin. The Mr 60'000 protein was shown to be a glycoprotein by concanavalin A binding. In the presence of benomyl and of tunicamycin at 0.5 µg/ml, two glycoproteins of lower molecular weight (Mr 54'000 and Mr 52'000) were secreted. The Mr 60'000 protein could be separated into two main and four secondary components by two-dimensional gel electrophoresis (pI = 6.67 and 6.52 and pI = 6.93, 6.81, 6.44 and 6.32, respectively). The Mr 60'000 protein was not a major intracellular protein of benomyl-treated cells and could only be revealed by immunoblotting with polyclonal antibodies raised against the extracellular form. It was undetectable in untreated cells collected at various stages of vegetative growth or in their culture medium.

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A short heat shock treatment removes the lag in alpha factor uptake in Saccharomyces cerevisiae

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After growth at 24°C in rich medium S. cerevisiae a cells internalize α-factor with biphasic kinetics. During the first 10 min the uptake rate is approximately 3 times slower than the rate after 10 min. This lag in α-factor internalization can be eliminated by treating the cells for 15 min at 37°C. This effect is probably due to induction of "heat shock proteins" as *cyr1* and *ras2* mutants, that constitutively induce some of these stress proteins at 24°C, show no lag. The role of the stress proteins may have something to do with transferring the α-factor receptor from its interaction with the signalling apparatus to the endocytic apparatus as a ste4 null mutant, that is unresponsive to pheromone, shows no lag in α-factor uptake at 24°C.

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EFFECTS OF C-TERMINAL MUTATIONS ON THE ENDOCYTOSIS OF YEAST α-FACTOR RECEPTOR

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The STE2 gene encodes the receptor for the yeast mating pheromone, α-factor, that has been shown to be endocytosed. Sequence analysis of STE2 suggests a membrane topology consisting of seven membrane spanning regions with the C-terminus in the cytoplasm. The importance of the cytoplasmic tail for proper receptor function has been demonstrated (1,2).
To test for a possible tyrosine-dependent endocytosis signal within this tail, the Tyr320 was mutated by oligonucleotide directed mutagenesis to Phe and Asn. In addition, to narrow down the region of the triggered endocytosis signal sequence, several truncations were created by introducing stop codons at position 312, 320 and 345. The uptake of α-factor by these mutated receptors is currently studied.

1)Konopka et al,Cell 54,609; 2)Reneke et al,Cell 55,221

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***ERD2*, an essential yeast gene required for the retention of luminal ER proteins**

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The C-terminal signal HDEL is both necessary and sufficient for the retention of luminal ER proteins in *S. cerevisiae*. We have isolated mutants that fail to retain their endogenous ER proteins. They define two genes (*ERD1* and *ERD2*) which have been cloned and sequenced. Here we describe *ERD2* an intron-containing gene encoding a 26 kDa membrane protein. Disruption of one chromosomal copy in a diploid strain produced a recessive lethal mutation. To verify this essential function a haploid strain containing this null allele and *ERD2* under GAL control was constructed. Repression of the GAL promoter with glucose arrested growth after 12h. Electronmicrographs of this conditional mutant showed an increase in the amount of ER membranes. Analysis of the wt protein sequence revealed an internal repeat of 18 aa of which 14 aa are conserved or analogous. These two repeats score as amphipathic helices in a computer analysis. Cloning one of the original UV-generated alleles of *erd2* by PCR, showed a mutation in this repeat. The change from Asp to Asn eliminates a negative charge and has a dramatic effect on HDEL retention. From this data and pulse chase experiments we conclude that *ERD2* has an important role in HDEL retention . Its molecular function in direct HDEL recognition and/or membrane traffic has to be established.
Supported by Roche Research Foundation.

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THE ROLE OF CHARGED AMINO ACID RESIDUES IN THE INSERTION PROCESS OF PROTEINS INTO THE MEMBRANE.

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We have studied the insertion of a small, 73 aa long protein into the cytoplasmic membrane of E.coli, the M13 procoat protein. As a first step the newly synthesized protein electrostatically interacts with the membrane. Removal of the positively charged residues (deletion) or exchange for negatively charged residues (substitution) results in a cytoplasmic location of the protein.
These mutant proteins also fail to bind to negatively charged liposomes in an in vitro assay. This suggests a direct interaction between the positively charged residues and the negatively charged phospholipids. In addition, in an E.coli mutant strain with a reduced amount of phosphatidylglycerol in the cytoplasmic membrane insertion of wildtype procoat was inhibited.
The positively charged regions of procoat remain at the cytoplasmic face of the membrane, only the negatively charged central region is translocated across the membrane. Substitution of the negatively charged by neutral residues does not prevent membrane insertion. A change into positively charged residues, however, progressively inhibits insertion. We assume that the positively charged residues interact with the cytoplasmic face of the membrane and thereby slowering the transfer of the protein region. The membrane insertion defect could be partially overcome in the E.coli mutant strain which lacks the negatively charged phosphatidylglycerol in its membrane.

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BINDING AND LATERAL DIFFUSION OF A SIGNAL PEPTIDE IN MODEL MEMBRANES

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Two different states of association of the fluorescence (NBD) labeled signal peptide of cytochrome c oxidase subunit IV with lipid model membranes have been detected by fluorescence spectroscopy, binding and lateral diffusion mesurements. The prevalence of a particular state depends on the presence of acidic phospholipids in the membrane. The peptide is loosly bound to bilayers of phosphatidylcholine where it diffuses rapidly in the choline headgroup region. In the presence of acidic phosphatidylglycerol, the peptide penetrates the bilayer more deeply and its rate of lateral diffusion is markedly decreased. It can be concluded that under these conditions, the peptide forms an oligomer of 3 to 6 partially helical membrane-spanning monomers, or (more likely), it inserts into the bilayer with its amphiphilic helical segment aligned parallel to the plane of the membrane and located near the headgroup and outer hydrocarbon region of the bilayer.

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A Novel Genetic Test to Probe the Topology of a Membrane-Protein

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Both HMG-CoA reductase isozymes of yeast *S. cerevisiae* are integral membrane-proteins and were predicted to contain seven membrane spanning domains. This study directly tested these predictions by fusing the yeast HIS4C domain, which encodes histidinol dehydrogenase, to specific sites in the isozymes. Yeast cells expressing the fusion proteins grew on histidinol-containing medium if the HIS4C domain was present on the cytoplasmic side of the ER membrane, but not if the HIS4C domain was targeted to the ER lumen. Biochemical analysis of the fusion-proteins confirmed the genetic results. The HIS4C domain of those fusion-proteins that conferred a His^- phenotype contained carbohydrate modifications and were thus present in the E.R. lumen.
Various transmembrane-domains were deleted from the fusion-gene and the behavior of the resulting protein was tested. In general deletion of an even number of putative transmembrane domains did not interfere with the protein's topology, but deletion or duplication of an odd number of transmembrane domains inverted the protein's orientation.
The described methodology should allow the topological probing of any yeast membrane protein.

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BINDING OF PROTEINS TO SPECIFIC TARGET SITES IN MEMBRANES MEASURED BY TOTAL INTERNAL REFLECTION FLUORESCENCE MICROSCOPY

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A new quantitative technique for measuring the binding of proteins to membranes is described. The method is based on a combination of total internal reflection microscopy and the preparation of supported planar bilayers. Specific and reversible binding of a fluorescence labeled monoclonal antibody to lipid haptens which were embedded in supported bilayers has been measured by this technique and compared to binding experiments which were conducted on membrane vesicles in solution. Equilibrium binding constants and kinetic parameters have been determined and used to expand the picture of the antibody-lipid hapten reaction. Estimates demonstrate that this technique is capable of measuring a broad range of binding constants (down to about 10^4 M^{-1}) using only small amounts of ligand and receptor.

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THE ER RETENTION SIGNAL OF THE E3/19K PROTEIN OF Ad-2.

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The E3/19K protein of human adenovirus type 2 is an abundant protein early during infection.Immediately after its synthesis it binds to human MHC class I antigens in the endoplasmic reticulum (ER).As the E3/19K protein is a resident of the ER, the complex formation with HLA antigens prevents their terminal glycosylation, cell surface expression and leads to a decreased T cell recognition of these cells.The E3/19K protein contains 142 amino acids and has the characteristics of a membrane protein: two carbohydrate groups are attached to asparagines 12 and 61, a putative membrane spanning segment is present 16-38 amino acids from the carboxy terminus.The ER retention signal of the E3/19K protein is contained within the 15 amino acids that protrude on the cytoplamic side at the carboxy terminus of the protein.We have by site-directed mutagenesis generated ten mutants of the E3/19K protein which differ from each other only within the short cytoplasmic tail containing the retention signal. Human embryonic kidney cells (293 cells) are analyzed after transfection with mutated E3/19K genes. Our results clearly show that a complex non-contiguous structure, consisting of three blocks of amino acids, constitutes the retention signal of the E3/19K protein.

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SORTING OF ENDOGENOUS PLASMA MEMBRANE PROTEINS OCCURS FROM TWO SITES IN CULTURED HUMAN INTESTINAL EPITHELIAL CELLS (CACO-2)

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The postsynthetic sorting of endogenous plasma membrane proteins was studied in a polarized epithelial cell line, Caco-2. Pulse-chase radiolabeling was combined with domain-specific cell surface assays to monitor the arrival of three apical and one basolateral protein at the apical and basolateral cell surface. Apical proteins were inserted simultaneously into both membrane domains. The fraction targeted to the basolateral domain was different for the three apical proteins and was subsequently sorted to the apical domain by transcytosis at different rates. In contrast, a basolateral protein was found to appear in the basolateral membrane only. Thus, sorting of plasma membrane proteins occured from two sites, the Golgi apparatus and the basolateral membrane. These data explain apparently conflicting results of earlier studies.

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SORTING OF HUMAN INTESTINAL AMINOPEPTIDASE N IN TRANSFECTED MDCK CELLS

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Apical membrane proteins are sorted via different pathways in different epithelial cell types. In MDCK cells apical proteins have been shown to be transported directly from the trans-Golgi-network to the apical domain whereas in hepatocytes and to some extent in enterocytes sorting via the basolateral membrane was demonstrated.
In the human intestinal cell line CaCo-2 it has been shown that a large fraction of newly sythesized aminopeptidase N (APN) is transported via the basolateral membrane to its final localization in the apical domain. In order to compare the sorting of an identical protein in cell lines showing different sorting pathways we have expressed human intestinal aminopeptidase N in MDCK cells. Roughly 80% was found in the apical membrane, 20% localized basolaterally. Sorting could be shown to occur directly. Currently we are investigating whether exchange between the apical and basolateral pool of APN takes place.

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A SINGLE MUTATION COULD BE RESPONSIBLE FOR ABERRANT GLYCOPHOSPHOLIPID (GPI) ADDITION TO CELL SURFACE PROTEIN AND LOWER LEVEL OF TRANSCRIPTION OF THE THY-1 GENE. N.Déglon, C.Bron and N.Fasel. Institut de Biochimie, Université de Lausanne, 1066 Epalinges.

The murine Thy-1 antigen present in significant amount on nervous tissues, thymocytes and peripheral T cells is attached to the cell surface via a GPI anchor. Thy-1 loss mutant lines have been isolated and these mutants synthesized the Thy-1 protein in normal amount but the antigen is blocked in the reticulum endoplasmic or actively secreted in the medium. In neither cases the GPI is added. In the "secreted" cell line, we show that the glypiation defect is not due to an alteration in the GPI signal but to a defect in the glypiation machinery. Futhermore in this cell line, we observed that the total amount of Thy-1 mRNA is reduced ca. 10x due to a lower rate of transcription of the Thy-1 gene. Both defects are corrected in somatic cell hybrids. A single mutation could be responsible for the two phenotypes. Isolation of Thy-1^+ revertant lines from this mutant thymoma in which the glypiation machinery and the level of expression of Thy-1 gene are restored would strongly support this hypothesis.

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REGULATION OF ENERGY METABOLISM VIA CONFORMATIONAL CHANGES OF MITOCHONDRIAL CREATINE KINASE (Mi-CK)?

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Mi-CK being involved in the maintenance of high ATP/ADP ratios is found in the mitochondrial intermembrane space of tissues with high energy demands. Two interconvertible oligomeric forms with different binding behaviours were found, dimeric and octameric Mi-CK. At slightly alkaline pH octameric, but not dimeric Mi-CK can be rebound to the inner mitochondrial membrane. On the other hand, Mi-CK can be released rapidly from the inner membrane (always as the octamer!), the release being followed by a slower dimerization of Mi-CK. This release of Mi-CK might abolish its functional coupling to oxidative phosphorylation and therefore diminish energy "production and transport". For the following reasons, conformational changes are thought to be the basis for the oligomeric interconversions, for the release and reassociation of Mi-CK from and to mitochondrial membranes, and thus for the regulation of energy metabolism: 1) In the case of chicken brain Mi-CK, a strict parallelism between release and dimerization of Mi-CK was observed. All conditions releasing Mi-CK from the mitochondrial membranes also lead to dimerization. 2) The dimer-octamer interconversion can not be the only basis for the regulation of energy metabolism, because in vitro, dimerization is observed primarily at low Mi-CK concentrations. At the concentration of Mi-CK calculated for the intermembrane space, octameric Mi-CK predominates even under "dimerizing conditions". 3) Conformational changes were already described for cytoplasmic CK isoenzymes.

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DIFFERENTIAL, pH-DEPENDENT ASSOCIATION OF Mi-CK DIMERS AND OCTAMERS WITH THE INNER MITOCHONDRIAL MEMBRANE

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Phosphate extraction of mitochondrial creatine kinase (Mi-CK) from freshly isolated, intact mitochondria of chicken cardiac muscle, after short swelling in hypotonic medium, yielded more than 90 % of octameric and only small amounts of dimeric Mi-CK. In extraction buffer, octameric Mi-CK displayed a tendency to dissociate, albeit at a slow rate with a half-life of approximately 3-5 days, into stable dimers.

Experiments with purified Mi-CK (octamers or dimers, or defined mixtures thereof) incubated under identical conditions with Mi-CK-depleted mitoplasts revealed that both oligomeric forms of Mi-CK are capable of rebinding to mitoplasts. However, the association of Mi-CK was strongly pH-dependent and, in addition, octameric and dimeric Mi-CK showed different pH-dependencies of rebinding. Therefore, it was possible under certain pH conditions to rebind either both oligomeric forms or selectively the octamers only. Furthermore, evidence was obtained that Mi-CK dimers partially form octamers upon rebinding to the inner membrane.

These results suggest that both oligomeric forms are physiologically relevant and that a change in the octamer to dimer ratio may be a factor influencing the association behaviour of Mi-CK in general and thus modulating mitochondrial energy flux as discussed in the PCr circuit model.

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FUSION AND LIPID TRANSFER BETWEEN INFLUENZA VIRUSES AND VARIOUS GD1a-CONTAINING LIPOSOMES

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As we previously demonstrated (Wunderli and Ott, Biochemistry 1990, in press) two distinct reactions could be kinetically defined upon interaction of GD1a-containing liposomes with influenza viruses: hemagglutinin-mediated fusion at low pH and lipid transfer at neutral pH. The two processes were quantified by measuring the dequenching of octadecylrhodamine B chloride (R18), a fluorescence membrane marker. Both are second order reactions, i.e. the interactions are collision-mediated. We now present results on the influence of the following parameters on these two processes: cholesterol content and lipid composition of the liposomes (in particular lipids with various transition temperatures), liposomal size (i.e. membrane curvature), detergent (residual amounts from liposome production in case of the detergent dialysis method), concentration of interacting particles and finally the incubation temperature.

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POLY (γ-GLUTAMIC ACIDS) ARE CRUCIALLY INVOLVED IN THE EXPLOSIVE EXOCYTOTIC PROCESS OF NEMATOCYST DISCHARGE

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Approx. 2 molar anions are constantly present in the lumen of nematocysts (capsular secretory products of stinging cells) of Hydra in situ. They make up the majority of the soluble capsular content and are organized as linear homopolymers of L-glutamic acids which are linked by γ-carboxy-α-amino amide bonds; the degree of polymerization is heterogeneous and dependent on the particular type of nematocyst. The polyanions are, in cooperation with corresponding cations decisively involved in the mainly osmotically driven exocytotic process of nematocyst discharge and contribute considerably to the generation of an intracapsular pressure of approx. 150 bar (Weber, J., 1989, Eur. J. Biochem. 184, 465-476).
This is the first time that poly (γ-glutamic acids), which are known to occur in some bacteria, are reported for eucaryotes. They may be present as predominant components also in nematocysts of other cnidarian species and thus might represent a class of compounds which is characteristic for a whole phylum of the animal kingdom (see also Tardent, P., Zierold, K., Weber, J., Gerke I.).

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METAL CATIONS IN THE NEMATOCYSTS OF CNIDARIA

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Upon an appropriate stimulus the cnidarian nematocysts discharge their tubular content by an explosive exocytotic process of evagination. In stenoteles of Hydra this process, during which the stylets perforate their target with an acceleration of 40 000 x g, takes less than 3 msec. The required forces are partly mechanical, partly due to an osmotically generated intracapsular pressure which can amount to 150 bar. Depending on either the species or the localization of the cysts the osmotically active components are either K^+, Ca^{2+} or Mg^{2+} and anionic poly-γ-glutamates. In isolated membrane free cysts the cations can be substituted experimentally by other cations (NH_4^+, Na^+, Co^{2+}, Fe^{2+}) without impairing the functionality of the cysts. According to X-ray microprobes performed with a STEM on ultrathin cryosections of undischarged and discharging Hydra stenoteles the K^+ (aver.conc. 2145 ± 737 mMol/kg dry weight) contained in the cyst remains within the cyst throughout the crucial phase of the process of discharge and is not released by the cyst until the long tubule is fully evaginated. During cnidogenesis the loading of the primordial cyst with $K^+$is associated with the invagination of the tubule assembled in the cytoplasm.

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REDOX CONTROL OF Ca^{2+} EFFLUX IN SYNAPTIC MEMBRANES

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The role of plasma membrane oxidoreductases (PMO) in the Ca^{2+} export mechanisms was studied in rat brain synaptic membranes. Both the high-affinity/low capacity Mg-dependent ATP-stimulated Ca^{2+} pump and the low affinity/high capacity ATP-independent Na^+-Ca^{2+} exchanger - wich control the Ca^{2+} efflux in nerve terminals - were strongly inhibited by pyridine nucleotides (PN), in the order NADP>NAD>NADPH>NADH with IC_{50}=10 mM for NADP and 3 mM other PNs (ATP-driven Ca^{2+} pump) and with IC_{50}=8 to 10 mM (Na^+-Ca^{2+} exchanger). Oxidizing agents such as DCIP and ferricyanide inhibited the ATP-driven Ca^{2+} efflux mechanism but not the Na^+-Ca^{2+} exchanger. Under conditions for complete activation of PMO, i.e. in the presence of PN together with oxidizing agents such as DCIP or ferricyanide, the inhibition of the ATP-driven Ca^{2+} pump was optimal, but the PN-mediated inhibition of the Na^+-Ca^{2+} exchanger was partially.

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CONSTRUCTION OF BACTERIAL EXPRESSION VECTORS FOR THE PRODUCTION OF PLASMA MEMBRANE CALCIUM PUMP FRAGMENTS

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The primary structure of several human plasma membrane calcium pump (hPMCA) isoforms has recently been determined as deduced from cloned cDNA (A. Verma et al., JBC 263, 14152-14159, 1988; E.E. Strehler et al., JBC 265, in press). These results together with direct protein sequencing, biophysical and computer modelling data led to the assignment of structural, functional and regulatory domains within PMCAs and suggest the existence of functional and regulatory differences between PMCA isoforms. In order to test for isoform-specific properties and to get a more detailed picture of structural and functional aspects of PMCAs it is essential to be able to generate and purify in sufficient quantity (fragments of) each individual isoform. cDNA fragments encoding the C-terminal putative regulatory domain of two hPMCAs were inserted into various prokaryotic expression vectors (pKK233-2, pTrc, pJLA502 and pIN-III-ompA) and expression of the foreign protein product was attempted under different conditions. The expression and accumulation of PMCA fragments was monitored on Western blots using specific antibodies and by the sensitive 125I-calmodulin overlay technique. Calmodulin binding PMCA fragments were produced at detectable but generally low levels in all systems tested. Surprisingly, however, the molecular weights of the major products did not correspond to the calculated value expected for the expressed products, indicating the formation of stable aggregates of the eukaryotic protein with itself or with as yet undefined proteins of E. coli. Large scale production of the PMCA fragments followed by purification by calmodulin affinity chromatography was performed for several constructs. Analysis of the products by peptide sequencing is currently underway.

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USE OF THE BACULOVIRUS SYSTEM FOR OVEREXPRESSION OF THE C-TERMINAL REGION OF PLASMA MEMBRANE CALCIUM PUMPS

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Two different isoforms of the human plasma membrane calcium ATPase (hPMCA) have recently been cloned and sequenced in our laboratory. The C-terminal region of PMCAs is of special interest because it contains the calmodulin binding "regulatory" region of these enzymes and accounts for the bulk of additional mass when comparing calcium pumps of the plasma membrane with those found in SR or ER membranes. To study and to compare the biochemical and regulatory aspects of the C-terminal portion of different hPMCA isoforms we are currently developing suitable systems for overexpression of these proteins. Such a system has now been found in the eukaryotic baculovirus expression system. Inside the insect host the baculovirus builds crystalline occlusions to provide viral protection. The major component of these extraviral aggregates is polyhedrin, a protein that is produced in large amounts. Taking advantage of the strong polyhedrin promoter, foreign genes can be expressed from recombinant baculovirus constructs in infected insect cell cultures. cDNA fragments coding for the C-termini of the two hPMCA isoforms were ligated into a transfervector that is easily propagated in E. coli and contains the regulatory sequences of the polyhedrin gene. The foreign sequences to be expressed were introduced into baculovirus by homologous recombination in vivo after cotransfection of insect cells with wildtype viral DNA and recombinant transfer vector DNA. "Positive" virus particles could be selected by agarose overlay assays since the recombinants produce plaques without occlusions. Calmodulin overlay and antibody assays (Western blots) show that the C-termini of the two calcium pump isoforms are indeed present and overproduced in cell lysates infected with recombinant virus particles.

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Ca^{++}-DEPENDENT ATPase ACTIVITY IS RESTRICTED TO THE APICAL DOMAIN OF THE PLASMA MEMBRANE IN PARATHYROID CELLS

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The cytoplasmic calcium concentration is about 10^{-7} mol, that of the extracellar space 10^{-3} mol. There is evidence that the Ca^{++}-dependent ATPase (Ca^{++}-ATPase) is directly involved in the maintenance of this gradient. Ca^{++}-ATPase activity was found in isolated membranes of parathyroid (PT) cells considering that a substantial proportion was bound to the plasma membrane, and a minor proportion to membranes of RER and mitochondria. After cytochemical demonstration of Ca^{++}-ATPase we found strong reaction products exclusively at the apical and lateral domain of the plasma membrane. Very weak reaction products were inconsistently found in mitochondria and RER. Replacement of Ca^{++} by Mg^{++} in the incubation medium demonstrated ATPase activity only at the apical and lateral plasma membrane domain. Incubation for demonstration (Na^+-K^+)-ATPase activity gave weak but distinct reaction products also at the apical and lateral plasma membrane domain only. Considering that the Golgi complex and secretory granules are located in the apical pole of PT cells, and that endocytic and exocytic processes were found only at the apical and lateral domain, these findings imply that many processes including ATPase dependent ion transport and secretion of parathyroid hormone take place exclusively at the apical and lateral domain of the plasma membrane.

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EXPRESSION OF SODIUM TRANSPORT AND Na, K-ATPase IN A CLONAL TOAD BLADDER CELL LINE.

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The toad urinary bladder cell line TBM 18-23 present, in vitro, many morphological and functional characteristics of well differentiated epithelial cells.

To correlate cytodifferentiation with the establishment of functional polarity, we examined the time course of morphological changes by electron microscopy and the establishment of sodium transport with cells seeded at low density on collagen-coated polycarbonate filters. Within 7-8 days, cells form a bilayer wich consist of upper typical granular cells and underlying microfilament-rich type cells, similar to those of the original bladder tissue. Functional polarity only appears when cells are morphologically differentiated (PD = 40-50 mV, R = 5000 Ω.cm2 at day 8).

We then were interested to follow the expression of the Na, K-ATPase genes during this cytodifferentiation process. In order to dispose of homologous nucleic probes and specific antibodies for the α and β subunits of the Na, K-ATPase of TBM cells, we constructed a cDNA library from size-fractionated poly A (+) RNA extracted from cells at day 8 of culture and screened this library with heterologous Xenopus laevis cDNA probes. We have cloned a full-length cDNA of 3.4 kb coding for a TBM α1 isoform. High AA homologies (85%) exist between TBM and Xenopus or mammals. Cloning of a β subunit cDNA is currently in progress.

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OLIGOMERIZATION IS A PREREQUISITE FOR STABILIZATION AND ER EXIT OF Na,K-ATPase α-AND ß-SUBUNITS

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Na,K-ATPase (NKA) is composed of a catalytic α-subunit (Sα) comprising all functional properties and of a glycosylated ß-subunit (Sß) of ill-defined function. In this study we have followed the fate of newly synthesized Sα and Sß in Xenopus oocytes injected with α and/or ß cRNA to examine whether assembly of the two subunits is needed for a correct folding and/or for intracellular transport of NKA. In oocytes injected with α and ß cRNA, the half-life of newly synthesized Sα and Sß increases significantly compared to oocytes injected with α or ß cRNA alone. In addition, Sß acquires complex-type sugars (indicative for translocation to the Golgi) only in the presence of concomitant synthesis of Sα. These data indicate that 1) assembly of Sα and Sß occurs at the level of the ER and 2) Sα and Sß mutually depend on each other to acquire a correct conformation likely to be necessary for their transport out of the ER.

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PUTATIVE $ß_2$-SUBUNITS HAVE SIMILAR FUNCTIONAL PROPERTIES AS $ß_1$-SUBUNITS OF Na,K-ATPase

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A putative $ß_2$-isoform of Na,K-ATPase (NKA) has been identified in cDNA libraries from neurula stages of X.laevis which shows about 60% homology to the renal Xenopus $ß_1$-subunit ($Sß_1$) (Good et al, in prep.). In this study, we have expressed $ß_2$ cRNA in Xenopus oocytes and have examined whether in analogy to $Sß_1$, $Sß_2$ is able to assemble to the catalytic α-subunit (Sα) and to form trypsin-resistant α-ß complexes. In oocytes injected with $ß_2$ cRNA and pulse-labeled for 4 hours, a polyclonal antibody against $Sß_1$ recognizes a newly synthesized 38 kDa coreglycosylated polypeptide which yields a 30 kDa non-glycosylated peptide upon endoglycosidase H digestion. After longer pulses, $Sß_2$ is processed to a 59 kDa ENDO H resistant fully glycosylated form. $Sα_1$ synthesized from injected $α_1$ cRNA becomes trypsin resistant in the presence of concomitant synthesis of $Sß_2$. These data suggest that $Sß_2$ as $Sß_1$ can confer stability to newly synthesized Sα, a process which is likely to be a first step in the functional maturation of the catalytic Sα.

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DEFECTIVE Na-TRANSPORT OF OUABAIN-RESISTANT RENAL Na,K-ATPase

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The rat renal Na,K-ATPase is resistant to cardiac glycoside inhibition. As it is not yet known whether the drug-resistance causes altered active transport, Na,K-ATPases from the renal outer medulla of rat (ouabain-resistant) and of rabbit (ouabain-sensitive) kidneys were reconstituted identically into liposomes. Purified renal Na,K-ATPase was used to rule out any influence of intracellular proteins. Enzymes of various specific activities were compared. The stimulation of the Na,K-ATPase activity by Na before reconstitution and of the active Na-transport after reconstitution was measured. Below 10 mM Na, we discovered a 50% Na-transport defect typical for the ouabain-resistant rat Na,K-ATPase. (Biochem. Biophys. Res. Commun., in press). The results demonstrate a link between the ouabain-sensitivity and the active Na-transport of Na,K-ATPase. SNSF31-25666.88

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CORRELATIONS BETWEEN ION FLUXES AND PROTEIN CONFORMATIONS OF Na,K-ATPase.

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The active transport of Na,K-ATPase (sodium pump) is associated with a crucial conformational transition of the alpha-subunit: E1/E2. The link between the ion movements and the conformational changes, however, is not yet known. Thus, the protein conformations induced by specific combinations of allosteric ligands and inhibitors of the pump protein are determined by differential trypsinolysis followed by quantitative analysis of the typical fragments by laser-densitometry. The ion movements associated with the conformations are measured in liposomes in which pure sodium pump molecules can perform active transport in the cellular as well as in the opposite direction. The predominant conformation appearing in active transport conditions (presence of Na,K,Mg,ATP) is E1. In the absence of ATP the Na,K-ATPase molecule induces a K-selective permeability associated with the E2 conformation. SNSF 31-25666.88

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SEARCH FOR FUNCTIONS OF HUMAN CALCIUM-BINDING PROTEINS

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Intracellular Ca^{2+} plays a central role in the regulation of various cellular processes e.g. the regulation of many enzymes or the control of membrane permeability. Of special interest are calcium triggered functions that are restricted to specific tissues (e.g. calcium dependent neurotransmitter release, neural excitability and plasticity in neurons or in muscles the involvement of Ca^{2+} in the contractile system and the intracellular signalling during the action potential).

To study the functions of calcium and calcium binding proteins (CBPs) we decided to use cultured human cells of muscle and brain tissues. Cell cultures allow expression studies by overerpression or producing anti-sense RNA of CBPs and functional assays. Towards this end we are first cloning full length human cDNAs of CBPs by screening human brain and heart cDNA libraries by various methods (screening of expression-libraries with antibodies, screening with vertebrate cDNA probes and with PCR).

Here we present as the first results of our efforts the isolation and cloning of calcium binding proteins (parvalbumin, calcyclin, S100α) of heart and brain.

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HOW INTRACELLULAR FREE CALCIUM IONS ACT ON SODIUM CHANNELS IN NIE-115 MOUSE NEUROBLASTOMA CELLS

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We previously reported that mouse neuroblastoma cells voltage-clamped in the whole-cell mode displayed a significantly higher macroscopic Na-current density with an intracellular free $[Ca^{2+}]$ of ≈100 nM compared with those investigated at less than 1 nM (Experientia 44:A70,1988). Now we show that current-voltage and steady-state inactivation curves taken together exclude surface charge phenomena as an explanation of the increased sodium current density. Recovery from inactivation of the channels due to the low resting potentials of the cells showed the same time dependency for 1 nM and 100 nM intracellular free calcium, and therefore we excluded this as a source of error. Furthermore, the change in membrane capacitance during experiments was not different for both intracellular free calcium concentrations and could similarly be excluded. Single Na-channel conductance as a possible mechanism of alteration of macroscopic I_{Na} was determined by means of non-stationary fluctuation analysis. For Na^+-concentrations of 120 mM outside and 12 mM inside the single Na-channel conductance was independent of the intracellular free calcium concentration and was found to be 9 to 11 pS at a temperature of 20° C. Thus more Na-channels seem to be activatable at 100 nM intracellular free Ca^{2+} than at 1 nM. (Supported by Swiss NF grant 3.143-0.85.).

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ACTIVATED RAS AND TPA BYPASS SYNERGISTICLY THE IL-3 GROWTH STIMULUS IN A MAST CELL LINE

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Expression of v-Ha-ras or of mutated c-Ha-ras (Val 12) in the bone marrow-derived mast cell line PB-3c leads to a reduction of the IL-3 growth requirement and to tumorigenicity. Expression of normal c-Ha-ras has no effect on the IL-3 requirement nor do the cells become tumorigenic. A transient reduction of the IL-3 requirement of the parental PB-3c can also be obtained by activation of Protein Kinase C using TPA. When the ras-expressing lines are treated with TPA a much more pronounced bypass of the IL-3 growth signal can be observed compared to the factor abrogation caused by either ras or TPA alone. Cell lines exhibiting a reduced IL-3 requirement do not show measurably elevated Protein Kinase C activity but they constitutively express high levels of c-fos.

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Inhibition of Gap Junctional Communication of galactose-phosphates by Retinoic Acid but not by Phorbol Ester TPA

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14C-galactose incorporated into cellular and secreted extracellular macromolecules by human fibroblasts is significantly reduced in Galactoskinase or galactose-1-phosphate transferase deficient fibroblasts. Coculture restores galactose incorporation by exchange of galactose-l-phosphate and UDP-galactose throug gap junctions between the two mutant cell types. Retinoic acid (RA) and the phorbol ester TPA are inhibitors of junctional communication by but by different mechanism. RA showed the expectet dose dependend inhibition of correction of galactose incorporation into cellular macromolecules in the cocultures. Some, although smaller inhibition of galactose incorporation into extracellular macromolecules was also found in the control fibroblasts. TPA seemed to inhibit galactose incorporation into cellular macromolecules in the cocultures in a dose independent way and also into the contol cells. Galactose incorporation into the secreted macromolecules (glycosaminoglycans) or the secretion of 14C-galactose labelled macromolecules were increased by TPA in all cell cultures.These effects interfered strongly with the inhibition of the gap junctional communication studied.

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ARACHIDONIC ACID MODULATES THE INTERCELLULAR COUPLING IN NEONATAL RAT HEART CELLS

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Cells were isolated from neonatal rat hearts and grown in tissue culture. De novo formed cell pairs were chosen to assess the conductance of gap junctional membranes (g_j) and channels (γ_j). The experimental approach used involved a double voltage-clamp method and whole-cell, tight-seal recording. Exposure to arachidonic acid (1-100 µM) caused complete uncoupling within minutes. The effects were reversible. Immediately before complete uncoupling and early during recovery from uncoupling, current signals arising from single channel activity were detected. γ_j was not affected by the concentration of AA. The analysis revealed a γ_j of 32 pS. Arachidic acid (100 µM) had no effect on g_j. Indomethacin did not prevent uncoupling by AA, thus ruling out an involvement of the cyclooxygenase pathway. Supported by SNSF (grant 31-25333.88).

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EFFECTS OF RETINOIC ACID ON INTERCELLULAR CURRENT FLOW IN CELL PAIRS OF NEONATAL RAT HEARTS

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Myocytes were isolated from neonatal rat hearts and grown in tissue culture. Spontaneously formed cell pairs were selected to determine the conductance of gap junctional membranes (g_j) and gap junctional channels (γ_j). The experimental approach adopted involved a double voltage-clamp method and whole-cell, tight-seal recording. Exposure to retinoic acid (RA; 1-100 µM) and retinol (10-100 µM) gave rise to complete uncoupling within 5-25 min. The effects were reversible. Immediately before complete uncoupling caused by RA and early during recovery from uncoupling, we observed current signals arising from single channel activity. γ_j was not affected by the concentration of RA. Analysis revealed a mean γ_j of 32 pS. Supported by SNSF (grant 31-25333.88).

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CELL SIGNALS INVOLVED IN THE CONTROL OF Na AND Cl TRANSPORT IN TRACHEAL EPITHELIUM IN CULTURE: POSSIBLE ROLE OF ARACHIDONIC ACID.

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The tracheal epithelium absorbs Na and secretes Cl. Cells isolated from the bovine trachea were cultivated. Monolayers grown in culture exhibited the same transport properties as the native tissue. In particular, Cl secretion was strongly stimulated by prosta glandins (PGE2). In this study the possible role of lipid signals in the control of ion transport was examined. Various concentrations of arachidonic acid (AA, 10-100 µM) enhanced conspicuously the short-circuit current (Isc) across monolayers (176 ± 15 % of stimulation, n = 3). Compared with the effect of a maximal dose of PGE2, AA induced a 93 ±4 % response. This effect was reduced to 43 ±6 % by a mixture of indomethacin (a blocker of PGs synthesis) and NDGA (nordihydroguaiaretic acid, an inhibitor of the lipoxygenase pathway), suggesting that AA itself may act as a second messenger in this system. Forskolin also increased the Isc, an effect that was additive to that of AA, in the presence of indomethacin and NDGA. This indicates the occurrence of separate pathways for AA and cAMP stimulations in these cells.

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DISTINCT SIGNAL TRANSDUCTION EVENTS LEADING TO HUMAN EOSINOPHIL RESPIRATORY BURST, SHAPE CHANGE AND DEGRANULATION

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Eosinophils from hypereosinophilic patients were stimulated with complement factor C5a, platelet-activating factor (PAF), neutrophil-activating-peptide (NAP-1/IL-8) and N-formyl-Met-Leu-Phe (fMLP). The respiratory burst, release of eosinophil peroxidase, shape changes and the rise in cytosolic free calcium concentration were measured. All agonist-induced responses were mediated via pertussis-toxin sensitive G-proteins. Depletion of intracellular Ca^{2+} prevented the respiratory burst and exocytosis but not the shape changes. Phorbol ester pretreatment restored the C5a- and PAF-mediated respiratory burst response of Ca^{2+}-depleted eosinophils. Inhibition of protein kinase C by staurosporine inhibited the respiratory burst totally, did not affect shape changes and increased the peroxidase-release. The activation of the respiratory burst in human eosinophils appears to occur by two parallel transduction sequences: One represented by Ca^{2+}-dependent activation of protein kinase C and the other by a receptor-mediated, Ca^{2+}-independent pathway.

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DIFFERENTIAL AMPLIFICATION OF ANTAGONISTIC RECEPTOR PATHWAYS IN NEUTROPHILS. Mueller,H., Weingarten,R., and Sklar,L.A. Res. Inst. of Scripps Clinic, La Jolla, CA 92037, USA.

In human neutrophils about 500 ligand-occupied beta-receptors are able to almost completely inhibit the superoxide production generated by at least 50,000 formyl peptide receptors, suggesting a massive amplification of the inhibitory receptor signals. We examined two stages of amplification. In the first stage, we have quantitated the ligand-dependent GTPase activities. For the formyl peptide receptor, the number of phosphates released from GTP in the presence of the saturating ligand is relatively modest, i.e. on the order of 1 per minute per receptor, eventhough there are about 200G_i (G_i type II) proteins per formyl peptide receptor in neutrophil membranes. In contrast, the number of GTPs cleaved in the presence of a beta-adrenergic agonist is about 100 per minute per beta-receptor and there are about 700G_s per beta-receptor in membranes. Thus the beta-receptor signal is already massively amplified at the G protein, whereas the signal of the formyl peptide receptor is likely to be amplified at subsequent steps. The second stage of amplification of the beta-receptor signal is the ouptut of the adenylate cyclase which was estimated by measuring the amount of cAMP necessary to inhibit response of electropermeabilized cells. Each receptor was estimated to generate 10,000 molecules of cAMP.

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A NERVE GROWTH FACTOR UNRESPONSIVE MUTANT PC12 CELL LINE - A TOOL TO STUDY SIGNAL TRANSDUCTION MECHANISMS OF NERVE GROWTH FACTOR

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The rat PC12 pheochromocytoma cell line has been widely used for the study of the mechanism of action of nerve growth factor (NGF). PC12 cells respond to NGF by ceasing cell division and acquiring neuronal properties including growth of neurites. It is well established that the biological actions of NGF are initiated by the specific binding of the protein to cell surface receptor molecules, but the mechanisms of signal transduction still remain poorly understood. A mutant PC12 cell line deficient both in short-term (c-fos proto-oncogene induction, S6 kinase activation) as well as long-term (increase in choline acetyltransferase activity, growth of neurites) NGF responses was examined for NGF receptor expression using a ^{125}I-NGF crosslinking/immunoprecipitation assay. Our results indicate that this NGF-nonresponsive cell line expresses truncated NGF receptor molecules still comprising the NGF binding site. Further analysis of this defective receptor should be of great value in characterizing the signal transduction mechanisms of NGF receptors.

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CNS MYELIN NEURITE GROWTH INHIBITOR PROTEIN CAUSES COLLAPSE AND RETRACTION OF RAT DRG GROWTH CONES BY CHANGES IN INTRACELLULAR CALCIUM

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Recent results have shown that two proteins present in oligodendrocyte membranes and in myelin exert a potent inhibitory effect on growing neurites (NI-35 and NI-250). Neutralization of these membrane proteins by monoclonal antibodies (IN-1) allowed growth on CNS myelin in vitro and regeneration of lesioned neurites also in vivo. We have now monitored the behavioral changes of individual growth cones and growing neurites from dissociated dorsal root ganglion (DRG) cells during the addition of NI-35 incorporated into liposomes, using time lapse video microscopy. 75 % of the observed growth cones collapsed and retracted within 8 min after NI-35 addition. In contrast, no responses were seen with control liposomes loaded with a non-inhibitory CNS myelin protein (70 kD), or with NI-35 liposomes preadsorbed with IN-1 antibodies. Fura-2 measurements of the growth cone calcium levels showed that these behavioral changes were correlated with an influx of calcium. These results suggest that the oligodendrocyte membrane constituent NI-35 inhibits neurite extension by raising intracellular calcium concentrations.

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SIMULTANEOUS MONITORING OF CYTOSOLIC FREE CA^{2+} AND EXOCYTOSIS AT THE SINGLE CELL LEVEL IN PITUITARY AND NEUROBLASTOMA CELLS.

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Rat pituitary cells in primary culture and neuroblastoma NCB20 cells were exposed to the fluorescent marker quinacrine to label secretory granules. Individual pituitary cells stimulated electrically by a depolarizing pulse applied with an extracellular electrode, responded with a lowering of fluorescence, corresponding to the loss of quinacrine from exocytosed granules. A similar response was seen in NCB20 cells stimulated with bradykinin. To look at cytosolic Ca^{2+}, $[Ca^{2+}]_i$, and exocytosis simultaneously, cells were loaded with fura-2 and quinacrine, and fluorescence monitored at λ_{ex}=380 nm (Ca^{2+} sensitive λ) and 360 nm (isosbestic λ, reflecting exocytosis). Alternatively, two λ_{ex} (354 and 380 nm) were chosen such that a rise in $[Ca^{2+}]_i$ produced no change in the sum $F(\lambda_{ex}1) + F(\lambda_{ex}2)$ for fura-2 alone. In doubly labelled cells, $F(\lambda_{ex}1) + F(\lambda_{ex}2)$ reflected exocytosis, whereas $F(\lambda_{ex}1)/F(\lambda_{ex}2)$ showed $[Ca^{2+}]_i$ changes. Exocytosis was only observed if $[Ca^{2+}]_i$ increased concomitantly. Only a fraction of the cells responding with a rise in $[Ca^{2+}]_i$ showed exocytosis. Re-stimulation often caused a rise in $[Ca^{2+}]_i$ without exocytosis.

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EFFECTS OF LIGHT ON PHOSPHOLIPID-TURNOVER IN RAT RETINA IN VITRO

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To investigate different aspects of the influence of light on the metabolism of retinal phospholipids we developed an in vitro incubation system for isolated rat retina. The precursors used for radioactive labeling of phospholipids were 3H-inositol and 14C-arachidonic acid. Retinas were incubated from one half hour to 6 hrs. There was a significant difference in the labeling of total phospholipids and of phosphatidic acid in light versus dark after 4 hrs. of incubation. Similarly, phosphoinositide turnover was stimulated by light.

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ANGIOTENSIN II INDUCED RENAL VASOCONSTRICTION IS ESSENTIALLY MEDIATED BY PROTEIN KINASE C ACTIVATION

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Using the isolated perfused rat kidney model we investigated the subcellular pathways along which angiotensin II (ANG II) exerts its constrictor effect on the renal vasculature. Renal vasoconstriction caused by ANG II (100pM) was greatly reduced by staurosporine (30, 100nM), a putative C-kinase inhibitor. Stimulation of C-kinase by phorbol-12-myristate-13-acetate (PMA, 1-100nM) caused a sustained and dose-dependent decrease of perfusion flow rate which was prevented by preceeding infusion of staurosporine. ANG II and PMA induced renal vasoconstriction was attennuated by the calcium channel blockers verapamil (5uM) and nifedipine (5uM). Our findings are compatible with the concept that ANG II induced renal vasoconstriction is essentially mediated by C-kinase activation. The activation of protein kinase C seems to require or to induce enhanced transmembrane calcium influx.

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INTERLEUKIN 1 (IL-1) AND TUMOR NECROSIS FACTOR (TNF) AMPLIFY ANGIOTENSIN II (AII)-STIMULATED PROSTAGLANDIN E2 (PGE2) SYNTHESIS IN RAT RENAL MESANGIAL CELLS

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In resting mesangial cells, AII, the calcium ionophor A23187 and the phorbol ester 12-O-tetradecanoylphorbol 13-acetate (TPA) stimulated PGE2 formation. After pretreatment with IL-1 or TNF, which are themselves potent stimuli for PGE2 synthesis, mesangial cells displayed an amplified response to AII, A23187 and TPA. The cytokine-induced effects occurred in a time- and dose-dependent manner and were attenuated by actinomycin D and cycloheximide. A potentiation in the response of cytokine-pretreated cells was observed with exogeneous arachidonic acid. In addition IL-1, TNF and AII increased the level of free arachidonic acid. These results suggest that IL-1 and TNF exert a priming effect on PGE2 production in mesangial cells.

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INTERLEUKIN 1 (IL-1) AND TUMOR NECROSIS FACTOR (TNF) SYNERGISTICALLY STIMULATE PROSTAGLANDIN E2 (PGE2) SYNTHESIS IN MDCK CELLS

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IL-1β time- and dose-dependently stimulated PGE2 synthesis. While TNF produced a comparatively small but significant stimulation of PGE2 release, co-incubation of IL-1β with TNF produced a marked synergistic stimulation of PGE2 release. The effect of IL-1β and of IL-1β and TNF was apparent as early as after 2 hours of incubation. The enhanced PGE2 synthesis was inhibited by indomethacin as well as actinomycin D, while cycloheximide surprisingly potentiated PGE2 synthesis in response to both IL-1β and TNF. IL-1alpha alone was ineffective in stimulating a significant release of PGE2 at concentrations as high as 10 nM. However, it too showed a marked synergistic interaction with TNF in stimulating PGE2 release.

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FSH BIOASSAY : METHODOLOGICAL ASPECTS.

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Pituitary hormone immunoreactivity (I) estimated by RIA does not always reflect hormone bioactivity (B). Jia et al (1985) developed a sensitive granulosa cell aromatase bioassay (GAB) claimed to detect FSH in normal serum. GAB is based on FSH stimulation of estrogen production from precursor androstenedione by cultured rat ovarian granulosa cells; total estrogen accumulation in the medium after 72 h is measured by RIA. For the assay, serum samples are pretreated with 12% PEG 8000 to reduce inhibitory factors (^{125}I-hFSH recovery is 93% after PEG pretreatment). In our hands however the original method failed to generate FSH dose-responses. We modified the procedure by varying the concentrations of two main additives in the cell cultures ($6x10^5$ viable cells/well), viz. phosphodiesterase inhibitor MIX 0.062 mM (vs 0.125 mM) and insulin 13.4 mU/ml (vs 26.8 mU/ml) while the others were unchanged (DES 10^{-7} M, hCG 400 mU/ml). Addition of TGF-B (1 ng/well) further improved GAB sensitivity. With these changes, we obtained dose-responses from 0.125 to 2.0 mU FSH/well (78/549 hFSH IRP), corresponding to serum concentrations of 3 to 50 mU/ml. This allowed us to measure FSH bioactivity in human serum and preliminary data display a FSH B/I ratio of 2 to 3 in normal individuals.

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DEVELOPMENT AND COMPARATIVE ANALYSIS OF A RADIORECEPTOR ASSAY AND TWO SOLID-PHASE RADIOIMMUNOASSAYS FOR HUMAN IL-1.

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We developed a radioreceptor assay (RRA) based on the competition between ^{125}I-rIL-1α (provided by CIBA-GEIGY) and standard or unknown quantities of IL-1α or β for binding to a limited amounts of IL-1 receptor (IL-1R). The source of IL-1R was a membrane preparation isolated from a subclone of EL4 mouse thymoma cell line. The ^{125}I-IL-1α bound to the membrane receptor was separated by magnetics beads coated with monoclonal anti-Thy-1 antibodies. This RRA detected 20 pg of IL-1 per 200 µl of human serum.
Results of the RRA were compared with those obtained with two recently developed solid-phase radioimmunoassays (RIA) for IL-1 using polyclonal anti-IL-1α or β antibodies coated to polystyrene balls as immunoabsorbent and ^{125}I - monoclonal anti-IL-1α or β antibodies as detector. The detection limit of these two RIA was in the range of 2 - 8 pg per 200 µl of human serum. Thus despite the high affinity of the membrane IL-1R (Ka = 5.3 x 10^{10} M) the RRA was not more sensitive than the solid-phase RIA. In addition, the RIA were able to discriminate between IL-1α from IL-1β, whereas the RRA was not. The RRA, however, allowed us to demonstrate the presence of an inhibitor in some normal human serum samples which is in the process of characterization. (Supported by Bühlmann Laboratories AG).

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AGE-RELATED ALTERATIONS OF PROLACTIN (PRL) BINDING SITES IN THE FEMALE RAT

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Aging is associated with various neuroendocrine alterations including in the rat a PRL hypersecretion with maintained ovulations (repetitive pseudopregnancies), a reduced activity of the hypothalamic dopaminergic neurons with loss of the neuron responsiveness to PRL suggestive of alterations in PRL receptors. We have investigated PRL binding sites in the hypothalamus, ovaries, mammary glands and liver of young (3-6 months) and old (26-29 months) pseudopregnant rats, using ^{125}I-oPRL and particulate membrane preparations. In the hypothalamus a negligeable binding of PRL was observed in all rats; in the ovaries the number of binding sites was similar in old and young rats, illustrative of a maintained luteotrophic effect of PRL with age. The number of binding sites was decreased with age in the mammary glands ($p<0.02$) while it was increased in the liver ($p<0.001$), supportive of the ability of PRL to induce its own receptors in this organ. The affinity constant of PRL binding was not altered with age whatever the tissue studied. These results are illustrative of tissue-specific alterations in the number of PRL binding sites with age and they are further supportive of multifactoriel regulations of these receptors.

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Expression of IGF II mutants with reduced type 1 receptor affinity

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The insulin-like growth factor II (IGF II) is a 7 kD polypeptide hormone with structural homologies to insulin and insulin-like growth factor I (IGF I). The in vivo function of IGF II is not known. We produced mutants of IGF II by site-directed mutagenesis. The aim is to obtain mutants which bind exclusively to the type 2 receptor and to avoid the crossreactivity to the type 1 receptor. We subcloned the cDNA into a mammalian expression vector and cotransfected into NIH 3T3 cells by calcium phosphate precipitation. Geneticin resistent cells were cloned and serum-free supernatant were tested by RIA and western-blots. Binding studies and biological assays will be the next step. Further IGF II was also expressed as a protein A-fusion protein in E. coli. The yield after CNBr cleavage and purification was 30µg/l.

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BIOSYNTHESIS OF LEUKOTRIENES IN RAT HEPATOCYTES

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Leukotrienes (LT's) are synthesized in cells of the myeloid lineage via the arachidonic acid cascade. The key enzyme 5-lipoxygenase exhibits dual enzymic activity by transforming arachidonic acid into 5-HPETE (5-hydroperoxy-eicosatraenoic acid) and the epoxide intermediate LTA4. LTA4-hydrolase and GSH-S-transferase (using GSH as a co-substrate) then convert LTA4 into LTB4 and LTC4, respectively. Although hepatocytes do not possess 5-lipoxygenase activity, they can, in absence of GSH, transform 5-HPETE into biologically fully active LTB4 (Gut, J., et al. Mol. Pharmacol. (1988) **34**, 256-264). We have extended these investigations and studied the profile of LT's synthesized by hepatocyte from 5-HPETE in presence of GSH. Increasing GSH concentrations led to a decrease in LTB4 formation and an increase in 5-HETE (5-hydroxyeicosatetraenoic acid) formation due to activation of enzymes with GSH-peroxidase activity. The presence of the precursor 5-HPETE caused a partial transformation of LTC4 to products whose structures are currently being analyzed.

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ALTERATIONS OF TSH ACTION IN THE THYROID GLAND OF OLD RATS

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Aging is characterized in rats by low T4 and T3 with unchanged TSH concentrations suggestive of an age impairement of TSH action on the thyroid gland. This view was evaluated by determining TSH binding to thyroid membranes of young and old male rats (4 and 24 months) as well as the activity of adenylate cyclase. Saturation analysis of ^{125}I-bovine TSH to thyroid membranes shows two types of binding sites similar in young and old rats, one of high and one of low affinity. The maximum capacity of TSH binding sites of both affinities are reduced by 40 % in old rats. The activity of adenylate cyclase in basal conditions is similar in old and young rats (1.0 ± 0.1 and 1.1 ± 0.1 nmol cAMP/2h/mg protein respectively), when stimulated by TSH (10 mU) it is significantly increased in young but not in old rats. In contrast GTP (2µM) or forskolin (10µM), two direct stimulators of adenylate cyclase, induce a similar increase in cAMP in young and old rats (200 % and 250 % respectively). These results suggest that the reduced density of TSH receptors with age is responsible for the low T4, T3 secretion. Post-receptor defects are not apparent since stimulations of adenylate cyclase activity by GTP and forskolin are as effective in the thyroid gland of young and old rats.

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THE ROLE OF LATERAL MOBILITY OF THE VASOPRESSIN V2-RECEPTOR IN CYCLASE-MEDIATED SIGNAL TRANSDUCTION IN LLC-PK1 CELLS

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Lateral mobility of the vasopressin renal-type V2-receptor was measured in the basal plasma membrane of cells of the LLC-PK1 porcine epithelial line using photobleaching and a rhodamine-labelled vasopressin analogue (TR-LVP). TR-LVP was shown to have binding properties and biological activities very similar to those of Arg8-vasopressin and could be used to fluorescently label the V2-receptor of living LLC-PK1 cells. LLC-PK1 cells incubated with TR-LVP in the presence of a 100-fold excess of AVP, or cells from the LLC-PK1 V2-receptor-deficient line M18 incubated with TR-LVP were used as controls for non-specific binding. The V2-receptor was largely mobile at 37°C; the mobile fraction (f) was about 0.9, and the apparent lateral diffusion coefficient 3.0 x 10^{-10} cm^2 /sec. V2-receptor mobility fell greatly with decreasing temperature; at 10°C f was reduced to 0.1. Activation of adenylate cyclase (AC) was studied in cells having reduced f of the V2-receptor, to test for a role of lateral diffusion of the V2-receptor in cyclase stimulation. This is the first in vivo investigation of the role of receptor mobility in AC-mediated signal transduction.

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MOLECULAR CLONING OF A NEW MEMBER OF THE SERINE/THREONINE PROTEIN KINASE GENE FAMILY

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Using a cDNA probe from the carboxy terminal region of the cAMP dependent protein kinase we have isolated two forms of a protein kinase from both human and porcine tissues. Analysis of these clones revealed a similar degree of homology (55%) to the catalytic domains of both protein kinase C and cAMP-dependent protein kinase and high homology between both α and ß forms from both species. Northern analysis showed a single band at 3kb expressed at varying levels in all the human cell lines and porcine tissues studied showing that both α and ß forms have a similar sized mRNA. Isolation of the 3' untranslated regions of the various isoforms may elucidate any tissue- or development-specific role of the different forms. An antipeptide antibody has allowed initial characterisation at the biochemical level.

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CHARACTERIZATION AND DYNAMICS OF PROLINE-DIRECTED PROTEIN KINASE ACTIVITY IN HUMAN HL60 PROMYELOCYTIC LEUKEMIC CELLS

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Recently a novel proline-directed protein kinase (PDPK) was described in rat pheochromocytoma. This serine/threonine protein kinase activity was also found in human promyelocytic leucemic HL60 cells. The PDPK from HL60 cells shows chromatographic characteristics essential identical to that of rat pheochromocytoma PDPK. The recognition site of the HL60 cell kinase displays an absolute requirement for a carboxy-terminal proline residue. PDPK phosphorylated histone H1 protein, glycogen synthase, and synapsin I, but not α-casein or ß-casein. Highest activity of the HL60 cell kinase was associated with or preceeded M-phase and decreased dramatically as HL60 cells underwent PMA-incuded differentiation. The major characteristics of the HL60 cell kinase appear to be identical to those of rat pheochromocytoma PDPK. Both are substantially different from those of other known cell division cycle (cdc) protein kinases.

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TWO PROTEIN KINASE GENES REGULATED DURING *Dictyostelium* DEVELOPMENT.

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Dictyostelium has a well described cAMP induced signaling pathway. However, no protein kinase genes implicated in transmembrane signaling have yet been described. The catalytic domains of mammalian protein kinases contain highly conserved regions. We have chosen one such region to deduce a DNA sequence using the Dictyostelium preferential codon usage, and to synthesize an oligonucleotide complementary to the coding strand. Two independent clones from a cDNA library have been isolated which encode putative serine-threonine specific protein kinases (Dd PK1 and Dd PK2). Dd PK2 shows over 50% sequence identity with the catalytic domains of mammalian and yeast cAMP dependent protein kinases. The N-terminal part of Dd PK2, however, appears unrelated to any known protein kinase. Dd PK1 shows about 40 % sequence identity with both protein kinase C and A. Both genes are expressed mainly 3 hours after the onset of starvation in *Dictyostelium*, and produce single mRNAs of 2.7 kb for Dd PK1 and 2.8 kb for Dd PK2.

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PERITUBULAR IgG DEPOSITS AND HYPERTROPHY OF FIBROBLASTS IN RENAL CORTEX OF RATS WITH ANTI-ERYTHROPOIETIN (EPO) AUTOANTIBODIES

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Although mRNA for EPO has recently been demonstrated in peritubular cells of the kidney cortex, the cellular production sites of EPO have not been identified. To obtain further information on the site of EPO formation, kidney morphology was investigated in 4 male Sprague Dawley rats immunized against EPO (2x60,000-100,000 IU recombinant human EPO/kg bw). Depending on the titer of antibodies cross reactive with endogenous rat EPO, animals developped different degrees of anemia (hct 48, 28,18 and 16%). Comparison of histology in anemic and non anemic animals revealed no change in glomeruli, tubuli and endothelial cells. However, kidneys of anemic animals showed marked hypertrophy of cortical interstitial fibroblasts, an increase in 5'ecto-nucleotidase activity of these cells and IgG deposits in the peritubular interstitium. These results confirm the role of the peritubular space as production site for EPO and indicate that fibroblasts might be involved in the formation of EPO.

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BLOCKERS OF BASOLATERAL K^+ CHANNELS IN A6 CELLS

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We have studied the properties of the basolateral potassium conductance of epithelial A6 cells grown on filters after treatment of the apical membrane with the pore-forming antibiotic amphotericin B. The electrophysiological transepithelial measurements were done under conditions where the short-circuit current (Isc) was a potassium current. Serosal addition of specific potassium channel blockers such as barium, quinidine or tetraethylammonium (TEA) reduced the potassium current. For these three blockers, the sensitive current was outward rectifying with equilibrium potentials of -76.3±0.9, -79.5±0.9, -78.5±0.5 mV, respectively. Barium block was concentration, voltage and time dependent and completely reversible. 5mM Barium inhibited 81.9±2.5% of the Isc. The half inhibition constant varied strongly with the membrane potential (from 115±12 µM at 0 mV to 8.2±1.4 µM at 120 mV).The block produced by 500 µM barium could be partly removed by membrane depolarization with a time constant of 0.92±0.09 s^{-1}at 0 mV. Quinidine block (86.9±0.8 %) was not completely reversible and had an inhibition constant of 9.8±0.9 µM, which increased when the membrane was hyperpolarized. 10 mM TEA inhibited irreversibly 38,6 % of the Isc.

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CLONING OF A PUTATIVE SUBUNIT OF THE EPITHELIAL SODIUM CHANNEL IN A6 CELLS

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An amiloride-sensitive sodium channel is localized to the apical membrane of high resistance epithelia such as the amphibian kidney cell line A6. Antibodies directed against the fusion protein corresponding to one cDNA clone isolated from an A6 expression library recognize the sodium channel purified from A6 cells and bovine kidney cells. The cDNA hybridizes to a mRNA > 5 kb from A6 cells and Xenopus oocytes. A 4.7 kb cDNA clone obtained from a Xenopus ovary library contains the complete coding region. Final proof that this cDNA contains the sequence of a subunit of the epithelial sodium channel will require the reconstitution of a functional channel.

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URATE TRANSPORT ACROSS THE BASOLATERAL MEMBRANE OF THE PIG RENAL PROXIMAL TUBULE

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Urate undergoes net secretion into the proximal tubule of the pig kidney. The basolateral step of urate secretion was investigated using membrane vesicles. An inwardly directed Na^+ gradient did not enhance the uptake of 42 µM ^{14}C-urate. But, in the presence of the Na^+ gradient, cis-addition of 150 µM 2-oxoglutarate stimulated the 1 min urate uptake to 143% of control (41,2 pmol/mg protein) and an overshoot of 124% was observed at 2 min. The cis-inhibition of various organic anions was tested on the 1 min urate uptake stimulated by 2-oxoglutarate and Na^+ gradient. In two experiments, 2 mM unlabeled urate and 1 mM probenecid inhibited 42 µM ^{14}C-urate uptake respectively by 28%/41% and 62%/68% while 2 mM lactate, pyrazinoate or p-aminohippurate did not produce any significant inhibition. These data indicate that, in the pig, urate might be translocated across the basolateral membrane by a mechanism similar to the one described in the rat for p-aminohippurate, that is a Na^+/dicarboxylate cotransport coupled to a dicarboxylate/anion exchange.

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PASSIVE DIFFUSION OF ^{14}C-SALICYLIC ACID THROUGH LLC-PK$_1$ CELL MONOLAYERS

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Monolayers of LLC-PK$_1$ cells which can be considered as a model of renal proximal cells were used to study the process of nonionic diffusion. We measured the apical-to-basolateral 4 min flux of salicylic acid (SAL). This flux corresponds to the reabsorptive flux which is probably passive in the nephron. LLC-PK$_1$ cells were grown to confluence on permeable collagen coated filters and unidirectional apical-to-basolateral permeability (P_{SAL}) as well as cellular content were measured at 20°C. The apical pH was varied from 6 to 7.4 basolateral pH being kept at 7.4. Cell content (pmol/µgDNA/10 µM apical) was 4.69±0.58 (n=6), 2.14±0.27 (n=6), 0.78±0.15 (n=6), 0.42±0.08 (n=6) and P_{SAL} (10^{-6}cm/s) was 24.4±1.46 (n=4), 9.16±0.50 (n=3), 6.13±1.36 (n=5), 3.83±1.23 (n=4) at apical pH of resp. 6, 6.5, 7.0 and 7.4. ^3H-Mannitol permeability, that represents the paracellular permeability did not vary with pH and averaged 1.37±0.43 (n=16). Thus, an increase in flux was accompanied by an increase in cell content, both being strictly correlated with the increase in non-ionized SAL.

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BIOLOGICAL ACTIVITY OF ELONGATED ANALOGUES OF OXYTOCIN (OT) AND VASOPRESSIN (VP)

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The group of Acher (PNAS, 86 : 5272, 1989) reported that elongated analogues of vasotocin elicit a hydrosmotic effect in amphibians, whereas they are devoid of uterotonic and vasopressor activity in rats. As an attempt to characterize epithelial amphibian receptors, we examined whether elongated analogues of OT and VP affected water permeability. Net water fluxes across bladders of toads Bufo marinus were recorded with an automatic technique. We found that OT 1-10 (OT-G) and OT 1-12 (OT-GKR) elicited a dose-dependent, hydrosmotic response at concentrations ranging from 10^{-9}M to 10^{-7}M. As shown before for VP, this effect could be inhibited by the barbiturate, methohexital, and enhanced by the flavonoid, quercetin. We found in addition that elongated analogues of VP (-G, -GK, -GKR) were even more potent, an increase in water permeability being detected already at 10^{-10}M. In conclusion, a hydrosmotic effect can be shown for non-amidated peptides that are precursor molecules of VP and OT.

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AMILORIDE-INDUCED INHIBITION OF WATER TRANSPORT

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We have previously shown in toad urinary bladder that amiloride (10^{-3}M) added to the serosal solution inhibits the hydrosmotic action of vasopressin (VP). In the hope of getting new insight on the mechanism of this inhibition, experiments were carried out in conditions where the VP-induced water flow was either increased (quercetin, high K^+, ouabain) or decreased (choline-Ringer). Amiloride significantly reduced the osmotic flow caused by 10^{-9}M VP in the presence of quercetin, high K^+ and ouabain by 35%, 36% and 41%, respectively. In choline-Ringer, the VP effect was reduced to 62% of that seen in Na-Ringer. Exposure to choline-Ringer and amiloride further reduced the VP effect to 34% of control. Two known targets of amiloride can be considered to explain these inhibitory effects : (1) the $Na/^+H^+$ antiporter ; (2) the adenylate cyclase. The effectiveness of amiloride in Na-free Ringers suggests that the inhibition of the hydrosmotic action might be mainly due to a reduction of cAMP generation.

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EARLY AND SODIUM-INDEPENDENT EFFECT OF ALDOSTERONE ON THE BASOLATERAL MEMBRANE CONDUCTANCE.

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Aldosterone induces a coordinated increase of the apical and basolateral membrane conductances in target epithelia. The mechanism responsible for the increase of the basolateral conductance is not known. We have measured transepithelial and intracellular potentials, and single membrane conductances in the epithelium formed by the TBM cells, an epithelial cell line coming from the toad urinary bladder. Aldosterone-treated (A) and control (C) epithelia were studied in paired experiments. Amiloride was added to the apical solution of aldosterone-treated cells to prevent the expected increase of the apical membrane Na^+ conductance; 40 to 600 nM amiloride were needed to maintain the sodium transport rate (measured as the short circuit current, I_{sc}) at the level measured in control. At 150 min after hormone addition, when amiloride was removed, I_{sc} had increased to 67 ± 10 µA.cm^{-2} (vs 43 ± 6 in control). The basolateral membrane conductance (mS.cm^{-2}) was 1.8 ± 0.3 in C, 3.2 ± 0.3 in A in the presence of amiloride and 3.0 ± 0.3 in A after removal of amiloride. Thus, aldosterone induced an early increase of the basolateral membrane conductance independently from any rise of the sodium transport rate or of the apical membrane Na^+ conductance.

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EXPRESSION OF RAT LIVER NA^+/L-ALANINE TRANSPORT IN XENOPUS LAEVIS OOCYTES. IN VIVO EFFECT OF GLUCAGON.

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Poly(A$^+$)RNA isolated from rat liver was injected into X.laevis oocytes. 4 to 6 days after injection of 40 ng of total mRNA, Na^+/Ala transport was stimulated 2,5-fold compared to water injected oocytes. The characteristics (kinetics, pH-dependency, substrate specifity) of the expressed Na^+/Ala transport suggest that both transport systems (type A and ASC) are expressed. By sucrose density gradient centrifugation, the mRNA encoding for Na^+/Ala transport was found to be of 1.9 to 2.5 kb in lenght.

Glucagon, known to regulate liver Na^+/Ala transport, was injected 4 hrs prior to mRNA isolation. Compared to control mRNA, mRNA from glucagon treated animals led to a 2-fold higher expression of Na^+/Ala transport. Na^+/Ala transport as expressed with mRNA obtained from glucagon treated animals, is encoded by the same size of mRNA as the control mRNA. It is concluded that glucagon modulates the abundance of liver-mRNA encoding for these amino acid transport systems.

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OVEREXPRESSION OF α-TYPE PKC IN PIG KIDNEY CELL LINE LLC-PK$_1$: EFFECT ON uPA mRNA INDUCTION AND EGF-RECEPTOR DOWNMODULATION

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Under uninduced conditions, the pig kidney cell line LLC-PK$_1$ expresses low levels of urokinase-type plasminogen activator (uPA). Addition of phorbol ester (TPA) results in a transient accumulation of uPA mRNA. In three stably transfected LLC-PK$_1$ cell clones overexpressing α-type PKC 5- to 20-fold, the extent of uPA mRNA induction by TPA is similar to the untransfected cell line. In the αPKC overexpressing clones, however, the accumulation of uPA message is significantly prolonged (2-3 fold) in a TPA dose dependent manner.
EGF binding to LLC-PK$_1$ cells is decresed to about 50% after 24h TPA treatment. This decrese in EGF binding is significantly more pronounced (10% to 20% EGF binding after 24h) in all αPKC overxexpressers in a TPA dose dependent manner.
The two reported effects coincide with the observation that the TPA induced PKC down regulation is markedly protracted in the αPKC overexpressing clones as compared to the parental cell line (as judged by Westernblot analysis with a polyclonal PKC antibody).

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Regulation of proximal tubular Na^+/H^+ exchange in monolayers of OK cells

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We have developed a method to measure the pH_i in confluent monolayers of OK (Opossum kidney) cells grown on plastic coverslips containing a small hole covered by a collagen coated permeant filter (Millicell) ("filterslips") by using the pH-sensitive dye BCECF(2,7-bicarboxyethyl-5(6)-carboxyfluorescein. The intracellular fluorescence ratio of the dye (F500/F445nm) was calibrated using the nigericin-high K^+ method. In the bicarbonate-free medium pH_i recovery from the acid (amonium) load is Na^+-dependent, with defined polarized distribution on the luminal side of the cells and its sensitivity to the presence of 5μM EIPA in luminal perfusate. The activity of the exchanger is increased at low intracellular pH and inactivated at high intracellular pH values.

We conclude that pH_i recovery measured in monolayers of OK cells is due to a presence of luminal Na^+/H^+ exchanger which is pH_i-dependent. Furthermore, it is possible to measure polarized distribution of Na^+/H^+ exchange by using a routine spectrofluorometer.

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A lipocortin like protein involved in adaptive regulation of Na^+/P_i transport in OK-cells.

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Adaption of renal proximal tubular Na^+/P_i-cotransport due to a low P_i-diet is mimicked in a cell culture model (OK-cells) by reducing the concentration of the extracellular phosphate. The observed increase of Na^+/P_i-cotransport is dependent on protein synthesis and is manifested by an increased Vmax. During the adaptive phase, cells were labelled with [^{35}S]-L-methionine and subsequently isolated plasmamembranes were analyzed by 2-dimensional gel electrophoresis. A two-fold increased incorporation of [^{35}S]-L-methionine was found in two proteins of M_r of 34 and 35 kD.
Microsequence analysis of the 35 kD protein resulted in peptide sequences homologues to the known sequences of the calcium/phospholipid binding proteins which belong to the lipocortin family. Highest homology was found to the lipocortin V. A possible role of the identified lipocortin like protein in the adaptive regulation of renal Na^+/P_i-cotransport is discussed.

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Apical Membrane Protein Phosphorylation in PTH Activation in OK cells: Participation of Protein Kinase A and Protein Kinase C.

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The present study was designed to evaluate the contribution of the cAMP/PKA regulatory system and the diacylglycerol/PKC system in PTH regulation in the proximal tubule cell model system, Opossum Kidney (OK). The method used was analysis of apical membrane protein phosphorylation patterns resulting from incubation with different PTH concentrations and by pharmacological activation of the PKA and PKC messenger systems. After 3 hr in situ incubation with ^{32}phosphate, monolayers were incubated for 10 min or 3 hrs with either two concentrations of PTH (10^{-10} or 10^{-8} M) or with 8 Br-cAMP or TPA to activate PKA or PKC, respectively. Apical membrane proteins were separated by 2-D page and analyzed by autoradigraphy. Different PTH concentrations resulted in different protein phosphorylation patterns suggesting different phosphorylation pathways function in PTH activation at different concentrations. Pharmacological activation suggested that low PTH is associated with PKC and high with PKA.

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CALCIUM AND PHOSPHATE TRANSPORT ACROSS THE JEJUNAL BRUSH BORDER OF AGING RATS

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Small intestine plays a key role in the homeostasis of calcium and inorganic phosphate (P_i). Intestine of young rats reabsorbs more calcium and P_i than in old ones. The brush border membrane appears to be the limiting step in the net reabsorption of these ions by intestine. The aims of this work are: 1. to investigate whether the transport of calcium and P_i across the brush border membrane of small intestine in old rats are lower than the rates in young animals, and 2. to characterize the transport of both ions across this membrane in aging animals. Brush border membrane vesicles (BBMV) were isolated from jejuna of young, adult and old Sprague-Dawley rats (3, 6 and 26 months old) and transport was estimated by a rapid filtration technique. Preliminary experiments show that Na^+-P_i uptake across BBMV from 6 and 26 months old rats is 50% lower than uptake in rats aged 3 months. Calcium uptake at 6 months was 30 % lower than uptake at 3 months and fell to 50 % of these values in the senescent group. Control experiments suggest that transport of calcium, rather than binding, was measured under our conditions. The transport processes for calcium and P_i will be characterized in kinetic terms in these rats. In conclusion, the reduction of net intestinal absorption of calcium and P_i observed in aging animals is expressed at the brush border membrane level.

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IN VITRO GASTROINTESTINAL UPTAKE OF URANIUM

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The mechanisms by which uranium and other actinides are absorbed from the gastro-intestinal tract are not yet understood. The main absorption probably takes place in the duodenum. Since in general the actinides are insoluble at neutral or alcaline pH, one can assume that complexing substances present in the stomach or duodenum at the time of ingestion of the actinides play an important role in determining the extent of absorption.
In our model we used NTA (nitrilotriacetic acid) as chelator and compared ferric iron with uranium uptake into brush border membrane vesicles. The results suggested facilitated transport of ferric iron as well as of uranium.

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ENTEROHEPATIC CIRCULATION OF SOMATOSTATIN-ANALOGUE PEPTIDES
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SMS 201-995, a octapeptide mini-somatostatin with a high potency against acromegaly and gastrointestinal endocrine tumours is absorbed as intact peptide in rat jejunum thereby reaching significant plasma levels as determined by a RIA-method. The absorption by brush border membrane vesicles of enterocytes was in part saturable and could be inhibited by structural analogues, indicating that uptake occurs not only by passive diffusion. By use of a fluorescent derivative of SMS we could demonstrate that the peptide is transcellulary transported by enterocytes of rat and hamster and hepatically extracted from portal blood. Similarly to other hexa to decapeptides the peptide interacts with hepatic bile salt uptake as shown by transport experiments with hepatocyte basolateral membrane vesicles and is secreted by the hepatocytes through the canalicular plasma membrane as demonstrated by fluorescent microscopy. During bile ductular passage a high accumulation of the peptide can be observed in the bile duct epithelial cells, whereas it's accumulation is rather low in the gallbladder epithelium.

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STRUCTURE OF THE HUMAN CD28 GENE

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The human CD28 protein is a 90 KDa -S-S- bonded dimeric cell surface molecule expressed on most T-cells with the exception of the T-supressor subset.In in vitro studies using anti-CD28 mAb as ligand, CD28 was found to function as a signal transducing element in T-cell activation. The investigation of the genomic structure showed the gene to consist of 4 exons. The exon/intron transitions have been sequenced.In order to investigate the strong inducibility of CD28 expression in T-cells 1.4 kb of the 5'flanking region have been sequenced. Only weak homology to known transcription factor binding elements were found. In CAT-assays constructs containing either 0.4kb or 1.4kb upstream sequences were investigated for inducibility. Preliminary data provide evidence for a regulatory element within this region.

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ISOLATION OF NON-VARIABLE ANTIGENS OF *TRYPANOSOMA* SSP.

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African trypanosomes are causative agents for trypanosomiasis affecting man, cattle and other animals. Much hope was placed in finding a vaccination against trypanosomal infections, based on the use of the variable surface glycoproteins (VSG's) as target antigens. Unfortunately a VSG based immunoprophylaxe has been ruled out due to the high antigenic variability of these proteins. Our project includes the identification of non-variable trypanosomal antigens by performing a differential immunological screening of a cDNA expression library of *Trypanosoma congolense*, which was constructed in the *Escherichia coli* vector Lambda gt11. These recombinant antigens will be expressed in live-attenuated *Salmonella typhimurium* carriers and subsequently investigated for their potential as immunoprotectants.

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Immune Intervention with Adjuvant Arthritis in Rats by a Nonapeptide from the 65 kD Mycobacterial Heat-Shock Protein

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Adjuvant arthritis (AA) in Lewis rats is a model of T-cell mediated autoimmune arthritis resembling human rheumatoid arthritis. A nonapeptide from the 65 kD heat-shock protein of M. bovis BCG, amino acid sequence 180-188, has been described to carry the dominant immunogenic epitope for both arthritis protective and arthritogenic T-cell clones. Immunizations with the synthetic nonapeptide completely protected 20 out of 26 rats against AA induced by M. tuberculosis. Interestingly, deletion of the N-terminal threonine of the nonapeptide resulted in loss of the protective activity. The protection was not due to the induction of tolerance to M.tuberculosis, however, a significant cellular immune response to the nonapeptide was observed. These findings suggest the feasibility of immune intervention using synthetic peptides in autoimmune arthritis.

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DIRECT INVOLVEMENT OF CD7 (gp40) IN ACTIVATION OF THREE TcR$\gamma\delta^+$ T-CELL LINES

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On three T cell leukemia lines, Peer Molt-13 and ICRF-1, expressing the $\gamma\delta$ T-cell receptor (TcR$\gamma\delta$), an other surface molecule CD7 (gp40) appeared to be involved in the activation process. Stimulation of these cells with a monoclonal anti-CD7 antibody (mAb) resulted in a rapid increase in cytoplasmic free calcium [Ca^2].

Activation through CD7 was further confirmed by measuring the production of IL-2 in ICRF-1 cells stimulated with an anti-CD7 mAb and the induction of mRNAs for TNFα, TNFβ and GMCSF in Peer and Molt-13 cells. The same anti-CD7 mAb was unable to activate TcR$\alpha\beta$ expressing Jurkat cells or normal resting peripheral blood T lymphocytes (PBL-T). In essence these findings contribute to favour the idea that on TcR$\gamma\delta^+$ cells, the CD7 molecule could play an important role during T cell differentiation.

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ACTIVATION OF $CD4^+$-T CELLS BY AN ANTI-CD4 mAbB66 INDUCES A SPECIFIC TRANSLOCATION OF MEMBRANE ENZYMES GENERATING SECOND MESSENGERS.

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PIP_2-Phospholipase-C and IP_3-Monophosphatase,key enzymes generating second messengers from phosphoinositides,were prevalently expressed in the cytosol of resting and stimulated human peripheral blood mononuclear cells. However, when lymphocytes were exposed to an anti-CD4 mAb B66 (1 ug/ml),a specific translocation of the membrane-associated activity of both enzymes occurred from the micro somal to the nuclear fraction of activated lymphocytes, accompanied by an increase of their specific activity .Since a comparable phenomenon was observed when cells were treated with PHA (10 ug/ml), which preferentially activates $CD4^+$-helper T lymphocytes, we suggest that activation and translocation of both enzymes to the nuclear compartment might be characteristic for the activation process of $CD4^+$-T cells.

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EXPRESSION OF HUMAN HISTOCOMPATIBILITY ANTIGENS ASSOCIATED WITH E3/19K IN INSECT CELLS.

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The human histocompatibility class I antigens (HLA) are expressed as heterodimers on all nucleated cells and have the function to present foreign peptides to T cells. The dimer consists of a heavy chain and a light chain called ß2-microglobulin. The E3/19K protein of human adenovirus type 2 associates to the HLA and retains the complex in the endoplasmic reticulum.
The search for an eukaryotic system deficient in an immune system similar to mammals, and in which overexpression of proteins can be achieved, led us to the baculovirus expression system.
Complementary DNA encoding the HLA-B27 antigen, the $ß_2$-microglobulin and the E3/19K protein were separately inserted into a baculovirus vector and recombinant virus was obtained. Infected insect cells were shown to produce the corresponding proteins in large amounts. In these cells we have studied the biosynthesis, glycosylation and intracellular transport. Particular attention has been paid to the assembly of the ternary complex and its influence on the cellular destination of the different subunits.

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HUMAN GLIOBLASTOMA CELLS RELEASE IL-6 IN VIVO

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Twenty glioblastoma cell lines, with one exception (LN-229), constitutively released IL-6 bioactivity as tested on an IL-6-dependent cell line 7TD1. A significant induction of IL-6 secretion was observed when the cells were treated with IL-1 or tumor necrosis factor-alpha (TNF). Various amounts of IL-6 mRNA were found in 5 of 6 cell lines tested. IL-6 mRNA was detected in line LN-229 only when the cells were treated with IL-1 or TNF confirming the bioassay data. Glioblastoma cells also produce IL-6 in vivo: 1) IL-6 activity was detected in 11/13 cerebro spinal fluids (CSFs) and 5/5 tumor cyst fluids. 2) IL-6 mRNA was found in 4/4 tumors. 3) Immunohistochemical analysis showed IL-6 within the tumor cells in 15/20 glioblastoma sections. In conclusion, the present study demonstrates for the first time the *in vivo* production of IL-6 by human glioma cells. We suggest that the elevated levels of serum acute phase proteins and immune complexes found in glioblastoma patients may be the result of this secretion.

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ISOLATION OF CLI, A NOVEL COMPLEMENT INHIBITOR, FROM HUMAN PLASMA AND SEMINAL PLASMA

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A novel human plasma protein designated CLI (complement cytolysis inhibitor) was isolated by affinity chromatography using a monoclonal antibody previously prepared against the soluble C5b-9 complex (SC5b-9). The purified CLI has a molecular mass of about 70 kd and consists of two nonidentical subunits of 35 kd, covalently joined by disulphid bonds. An allmost fullenght cDNA clone of 1.7kb was isolated from a human liver cDNA library. The encoded aa sequence consists of 427 aa residues preceded by a 21 aa long signal peptide. The two subunits of CLI are generated by posttranslational cleavage of the single chain precursor protein. CLI shows a high overall aa homology to rat SGP-2 and sheep Clusterin, two major products of Sertoli cells. Plasma levels of CLI were up to 100 ug/ml. Functional studies showed that purified CLI is a potential inhibitor of the C5b-7 initiated hemolysis by most likely interfering with the lipid insertion of nascent C5b-7. During serum activation with Zymosan, CLI is incorporated into the SC5b-9 complex. In addition, CLI was purified as a major protein from human seminal plasma (up to 15mg/ml), where it could play a crucial role in the protection of sperms against complement attack in the male and female reproductive tract.

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NUCLEAR INCLUSION BODIES (NIB) IN HUMAN LYMPHOKINE ACTIVATED KILLER (LAK) CELLS

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Recently peculiar NIB have been found in LAK cells obtained by in vitro cultivation of mononuclear cells in the presence of interleukin-2 (IL-2). In order to get more information on these structures we examined ultrastructure, frequency, IL-2 dependence, phenotype and killing properties of NIB bearing LAK cells. The NIB were formed by a complex network of membrane tubules which appeared to be derived from trapped ER profiles. They were completely separated from the cytoplasm as revealed by serial sections. The number of NIB bearing LAK cells increased during the cultivation period from 2% at day 6 to 12% at day 13. The expression of NIB was clearly dependent from IL-2 dose but not from serum supplements. Immune electron microscopic studies revealed that all NIB containing cells have CD3 molecules but that only 50% bear HNK-1 markers. LAK cells with NIB were found to bind readily to the appropriate targets indicating the intact lytic activity of the killer cells.

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Stability of antigenic peptides in serum.

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The use of peptides is of general and practical interest in immunology and medicine. However, one drawback that one might expect in using peptides in vivo is their relative instability in serum. We analyzed under different conditions the degradative effect of serum on several peptides that are recognized by T cells in association with the MHC (major histocompatiblity complex) class I or class II molecules. Peptides at different concentrations were placed at 4°C or 37°C in foetal calf serum or mouse serum for different lengths of time after which their biological activity was determined in either a cytolytic or proliferative assay using specific T cell clones. We have found that while certain peptides are very stable, others are easily degradable. In certain cases we were able to inhibit peptide degradation by specific enzyme inhibitors (as determined by HPLC), thus facilitating the identification of labile peptide bond(s) and the design of peptide analogs with increased stability toward enzymatic degradation.

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SIGNAL FOR ADDITION OF A PHOSPHOLIPID MEMBRANE ANCHOR ON THY-1 GLYCOPROTEIN.

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Many members of the Ig supergene family involved in homophilic and heterophilic cell-cell interactions (eg. $N\text{-}CAM_{120}$, LFA-3) as well as in cell activation and proliferation (Thy-1, Ly-6 E/A) are anchored in the cell membrane via a phosphatidylinositol-containing glycolipid (GPI). The amino acid signal which directs the addition of the GPI anchor to a specific cell surface protein is not known. However, the presence of hydrophobic carboxy-terminal sequence on the precursor polypeptides of the GPI anchored molecules and removal of this C-terminal segment upon maturation are essential elements for GPI-anchoring. Thy-1 has been shown to be GPI-linked. To investigate the amino acid structural requirements for GPI-anchoring, we introduced a number of mutations in Thy-1 carboxy-terminal region and studied their effects on Thy-1 processing and GPI anchoring. Our results demonstrate that two regions are necessary signals for the GPI addition: a stretch of hydrophobic amino acids involved in the protein-membrane interaction and a specific conformational motive recognized by the glypiation machinery.

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CYTOKINE REGULATION OF INTERCELLULAR ADHESION MOLECULE 1 (ICAM-1) EXPRESSION ON HUMAN GLIOBLASTOMA CELLS.

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ICAM-1 has recently been identified as one of the ligands for lymphocyte function associated antigen 1 (LFA-1). Immunohistochemical staining of frozen tissue sections using the ICAM-1 antibody RR1/1 demonstrated its expression also on human glioblastoma cells and on intratumoral vascular endothelial cells. ICAM-1 was weakly expressed or absent from low grade gliomas and absent from normal and fetal brain. ICAM-1 expression was similar to that of MHC class II, HLA-DR antigens. Glioblastoma cell lines constitutively expressed ICAM-1. Surface ICAM-1 expression and mRNA could be increased by preincubating the cells with IL-1β, TNF-α (1-10 U/ml) and IFN-γ (500 U/ml). IL-2, IL-4, IL-6 and TGFβ2 had no effect. Up to a 500 fold induction of ICAM-1 mRNA steady state level was observed 4h after cytokine treatment and decreased by 24h, whereas surface antigen expression of ICAM-1 increased for up to 48h.

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STUDIES ON THE ADAPTIVE RESPONSE OF HUMAN BLOOD LYMPHOCYTES TO X-RAYS.

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Whole blood from healthy donors was maintained in 9.0 ml of RPMI 1640 medium (20% FCS ± 2% PHA-M) solution. A 10mGy "adaptive" dose of x-rays was given at a specific time followed by a 1Gy "challenge" dose. Later the cells were exposed to colcemid, treated with a hypotonic solution and fixed. The cells were stained with giemsa and counts of chromosome aberrations made. It is known that a predose of x-rays can desensitize cells to a second dose (Fan et al., 1989). These studies show it is only those cells in G2 which demonstrate this reduction in damage and suggest a factor is present in the medium, released from adapted cells, which can induce this adapted state in non-exposed cells as inferred from dosimetric considerations (Burkart and Vijayalaxmi, 1988). These studies also demonstrate an increase in radiation damage with post-irradiation time if the cells are not permitted to pass through the cell cycle.

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DIRECT FLUORESCENT ANTIBODY LABELLING OF Ia ANTIGENS IN MURINE SMALL INTESTINE

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Major histocompatibility complex possesses genes coding production of membrane glycoproteins, i.e., Ia. The Ia antigens (Ag) are expressed on baso-lateral membrane of enterocytes, cell surfaces of activated B and T lymphocytes, and macrophages. Specimens from small intestines of six-week-old, C3H mice of K-haplotype were obtained, flushed, frozen and embedded. Frozen sections, 4-6 µm, were used. Fluorescein isothoicyanate conjugate monoclonal antibodies MRC OX-6, directed against mouse Ia Ag (10 µl/million cells), were added to sections that were incubated at room temperature for 30 min, mounted in FA mounting medium, and examined with a Fluorescent microscope. Enterocytes did not give a positive reaction. Numerous cells in the propria that were positive for class II Ag including macrophages acted as control. Conclusion: this method is not sensitive for demonstration of Ia Ag on enterocytes.

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FACS ANALYSIS REVEALS Tn- AND SIALOSYL-Tn ANTIGENS ON LYMPHOBLASTOID CELLS FROM A PATIENT WITH GALACTOSYLTRANSFERASE DEFICIENCY

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Expression of the Tn antigen due to galactosyltransferase (GT-)-deficiency on erythrocytes from a patient (R.R.) with permanent mixed-field polyagglutinability remained stable for 17 years. The enzyme from normal cells catalyzed the transfer of galactose (gal) to the glycan moieties of Tn membranes. Incorporation of gal was enhanced after neuraminidase treatment indicating the existence of the sialosyl-Tn antigen. Tn membranes were further characterized by the binding of anti-Tn and anti-sialosyl-Tn monoclonal antibodies (MAB). They were not stained with an anti-T MAB. A lymphoblastoid cell line was established from this individual by in vitro infection of his B lymphocytes with Epstein-Barr virus. When this cell line was stained with the anti-T MAB and analyzed by flow cytometry the profile was bimodal indicating the existence of two discrete populations. In additional experiments, 36% and 28% were stained with anti-Tn and anti-sialosyl-Tn, resp., while 38% were positive with anti-T. In comparison, 70 % of lymphoblastoid cells from a normal donor reacted with anti-T. 23% and 12% of the same cells were positive with anti-Tn and anti-sialosyl-Tn, resp.

These data indicate that B lymphocytes from R.R. are also affected by GT-deficiency. Cloned Tn lymphoblasts will be a useful tool to study O-glycosylation. This study was supported by grant 3.136.088 of the SNSF.

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HUMORAL IMMUNE RESPONSE AGAINST NATIVE AND DENATURED PROTEIN ANTIGEN

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We are analyzing the immune response against two antigens which are of identical amino acid sequence but of different spatial structure. The antigens compared are native yeast iso-1 cytochrome c (cyt c) and the heme-free yeast iso-1 apo-cytochrome c (apo-cyt c). The latter has no regular secondary structure; its CD-spectrum conforms to a random coil. We produced 30 monoclonal antibodies (mca's) and several polyclonal rabbit antisera against both forms of the antigen. Mca's and antisera were characterized by ELISA using as antigens several species-specific cytochromes c and 103 nested hexapeptides covering the entire sequence of the protein antigen, i.e., hexapeptides 1-6, 2-7 etc. We predicted that the denatured antigen, because it is a random coil, induces more peptide-reactive antibodies. This was not the case. The "surface-exposedness" in cyt c crystals of the immunoreactive peptides has been compared. Both antigens induce some heteroclitic mca's which react preferably with the opposite form of the antigen than that used for immunization.

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EPITOPE MAPPING OF ANTI-HUMAN IFN-γ RECEPTOR mAbs.

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The human interferon-γ receptor (hulFN-γR) is a novel 472 amino acid transmembrane polypeptide chain with about equally large extracellular (N-terminal) and cytoplasmic domains (Cell 55:273, 1988). Expression in mouse cells mediates high affinity binding, but is not sufficient to confer sensitivity to hulFN-γ, suggesting that additional species specific signal transducing elements are needed. To further elucidate the structure and function of the hulFN-γR a series of anti-receptor mAbs was produced using highly purified receptor protein as immunogen. Various cDNA fragments of the hulFN-γR were expressed in E. coli and used to narrow down epitope locations. 8 distinct epitopes could be distinguished, 4 were located on the extracellular part, 4 on the cytoplasmic domain of the receptor protein. mAb A6 strongly inhibits receptor binding of hulFN-γ and, therefore, the corresponding epitope is probably related to the ligand binding site. mAb 11B2 recognizes a cytoplasmic epitope. Upon microinjection this antibody was shown to neutralize hulFN-γ mediated expression of MHC class II antigens, indicating that the epitope could be involved in signal transduction.

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Egg yolk antibodies against small amounts of a conserved mammalian protein produced in chicken.

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The egg yolk of immunized chickens is a convenient and inexpensive source of specific polyclonal antibodies. Six laying chicken were immunized with different amounts of purified native proliferating cell nuclear antigen (PCNA), a highly conserved mammalian protein. PCNA is a cell cycle regulated protein which functions as an auxiliary subunit of DNA polymerase δ and plays a major role in eukaryotic DNA replication. The antibodies against PCNA were purified from the egg yolk by polyethylene glycol precipitation followed by DEAE and FPLC MonoQ chromatography. Immunoblot analysis showed that a total amount of 20-30 μg antigen was enough to induce an immune response after 20 days. Up to 72 mg antibodies per egg were isolated at least until day 81 after the first injection of the antigen. Due to immunization of laying hens with low amounts of the antigen it is possible to produce a large quantity of antibodies, even against highly conserved proteins that are slightly immunogenic in mammals.

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INTERACTION BETWEEN HALOTHANE AND THIOPENTAL ON RAT HEART

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With Langendorff's heart preparation of rats the influence of halothane (H) upon thiopental (T) uptake into heart tissue and its consequences on heart function were studied. Tyrode-solution (gassed with carbogen, pH 7.4) containing either T ($3-7 \cdot 10^{-5}$ g/ml) or T and H (0.8 - 2.0 vol%) was used as perfusion fluid. In the presence of H the T-concentration in heart tissue was significantly increased, a pharmacokinetic interaction already seen in experiments with rats *in vivo* (Büch et al., Naunyn-Schmiedeberg's Arch Pharmacol 337 N° 22, 1988). Heart rate, contractility and coronary flow were decreased dose-dependently by T in the Langendorff heart preparation. In contrast to the H caused increase of T uptake into heart tissue the negative chronotropic action of T was reduced by simultaneously present H whereas the negative inotropic action remained unaffected. When using isolated right and left atria, both, the negative chronotropic and inotropic action of T were potentiated by the presence of H.

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CHEMICALLY INDUCED ALTERATIONS IN RAT HEPATOCYTE CULTURES MONITORED BY TWO PARAMETER DNA/PROTEIN FLOW CYTOMETRY.

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Hepatocyte cultures were established from young adult rats. Cultured cells were exposed to phenobarbital, dimethylsulfoxide, thioacetamide, cyproterone acetate and retinoic acid from day 1 to day 3-4. Single cells isolated from monolayer cultures were subjected to a two parameter DNA/protein flow cytometry analysis. Alterations examined were: 1) contribution of 2C, 4C, and 8C cells, 2) cellular protein content and 3) number of binucleated cells. The method allows the influence of the hepatocyte isolation procedures (Percoll gradient), single cell recovery (trypsin resistance), culture dishes and alterations occuring with increasing culture time to be monitored at a cellular level. Among the 4C hepatocytes, which contribute to more than 60% of the cultured cells, two subpopulations were identified. Each of them accumulates in number and in cellular protein content after treatment with the test agents. In addition, the chemicals increased the number of binucleated cells. It is speculated that the two subpopulations represent functionally different hepatocytes and are therefore suitable indicators for the characterization of hepatotoxic activity of chemicals in vitro.

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DETECTION AND CHARACTERIZATION OF ANEUPLOIDY INDUCING CHEMICALS IN V79 CELL CULTURES BY FLOW CYTOMETRY.

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Proliferating V79 Chinese hamster cells, were exposed during 24 hours to agents known to induce aneuploidy in yeast (phenobarbital, cholic acid, bavistan, nocodazol) or in mamalian cells (estriol) After a 18-24 hours culture period without the chemicals, cellular protein and DNA content were analyzed by flow cytometry. Cell-cycle phases (G_0/G_1, S, G_2/M) and coefficient of variations (CV) were calculated. The contribution of metaphase (M) cells was determined by a separate flow analysis (propidium iodide/Höchst method). At highest exposure levels, phenobarbital (4-10mM) promotes 10-30% of the cells into a second cell-cycle to the tetraploid stage. Bavistan (10-100 µM), nocodazol (0.025-0.1µM), cholic acid (1-3mM) and estriol (50-100µM) arrested cells dose dependtly at the G_2/M cell cycle phase. With bavistan and nocodazol the cells in the G_2 peak represented M-phase cells. The combined analysis of cell cycles and contribution of metaphase in the G_2/M cells seems to be a promissing method to detect and characterize aneuploidy inducing agents in cell cultures.

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COMPARATIVE EFFECTS OF CAFFEINE, PARAXANTHINE, THEOPHYLLINE AND THEOBROMINE ON THE SPONTANEOUS ACTIVITY (SA) IN THE RAT

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Paraxanthine, the main caffeine metabolite in man but not in animals, has been the subject of few investigations. To evaluate the relative potency of the dimethylxanthines present in plasma after caffeine administration, male rats received at 8 A.M. either saline or one of these methylxanthines orally at doses ranging from 0.5 to 64 mg/kg. SA was automatically registered each 15 min. for 3 hours. A 4-fold increase in SA was observed for theophylline (8mg/kg), the most potent methylxanthine, while caffeine and paraxanthine gave only a 2-fold increase. SA stimulated by theophylline reached a maximum value at 32 mg/kg followed by a decrease at higher doses. Maximum SA for caffeine was reached at 16 mg/kg but was only half that of theophylline. On the contrary, SA increased continuously with the dose of paraxanthine, being equipotent to theophylline at 32 mg/kg. No stimulant effect could be demonstrated for any dose of theobromine. These results demonstrate the important contributions of theophylline and paraxanthine to the stimulation of SA attributed in vivo to caffeine and the need to evaluate their presence in plasma when extrapolating results from animals to humans.

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SIX ANTIVIRAL AGENTS TESTED FOR GENOTOXICITY IN THE SOMATIC MUTATION AND RECOMBINATION TEST OF DROSOPHILA

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Genotoxic agents can be identified by means of the somatic mutation and recombination test of Drosophila. This test is based on the recessive wing cell markers mwh and flr. Mutation as well as recombination lead to mutant clones in a wildtype background on the wings of flies which are trans-heterozygous for the markers. Antiviral agents were tested for genotoxic effects in this test system: (1) The anti-AIDS drug 3'-azido-3'-deoxythymidine as well as 2',3'-dideoxy-cytosine lead to 3' DNA synthesis gaps and are genotoxic in this way. (2) Genotoxic effects were also found for acyclo-guanosine (acyclovir) which as acyclo-GTP can compete with GTP and thus inhibits DNA polymerase. (3) Ribavirin competitively inhibits early steps in guanosine monophosphate synthesis; its genotoxic effects are most likely due to the resulting imbalance in the nucleotide pool. (4) Two adenosine analogs, tubercidin and formycin A, are presumably non-genotoxic as inferred from negative preliminary test results.

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STIMULATION OF PYRIMIDINE PHOSPHORYLASE AND SYNERGISTIC EFFECTS OF 5'-DEOXY-5-FLUOROURIDINE WITH INTERFERON α.

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The biological effect of 5'-deoxy-5-fluorouridine (DFUR) depends upon its intracellular enzymatic cleavage by pyrimidine phosphorylase to form fluorouracil (5FU). Among other pyrimidine derivatives, this prodrug exhibits a high therapeutic index. When tested in combination with interferon α (IFNα) on the proliferative activity of 8 human colon cancer cell lines, a synergized inhibition has been obtained with DFUR but not 5FU in 4 lines. In these lines but not in the others, we show that cell extracts contain increased pyrimidine phosphorylase activity after 5 days of exposure to IFN α.
Pyrimidine phosphorylase activity being significantly higher in tumoral tissues vs normal counterparts, it appears that IFN α may well enhance DFUR therapeutic index by potentiating the antitumoral activity of this drug.

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EFFECT OF THE DOPAMINE (DA) ANTAGONIST SULPIRIDE (S) ON A VISUAL PERCEPTIVE FUNCTION, VISUAL MASKING, IN VOLUNTEERS

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Physiological studies suggest that DA may be an important transmitter in the retina. Despite wide use of DA antagonists in psychiatry, little effects of these drugs on visual perception have been reported. We assessed the effects of a single dose of the presynaptic D_2-antagonist S (100 mg i.m.) on backward masking. In a tachistoscope a target letter (t)followed by a mask (m) composed of chopped letters was presented. The independent variable was the temporal interval between the onset of the t and m (SOA). In 6 volunteers, 3 h after dosing, a 100% increase of misidentifications of t was found at SOA of 60 ms. Log-lin analysis indicated a strong decrease of performance ($p>0.0001$). The effect of S on spatio-temporal aspects of visual information processing may be of relevance for everyday situations such as driving a car.

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EXPRESSION OF HUMAN MICROSOMAL EPOXIDEHYDROLASE IN SACCHAROMYCES CEREVISIAE AND ITS USE IN THE STUDY OF DRUG INTERACTION BETWEEN EPOXYCARBAMAZEPINE AND VALPROMIDE

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The human microsomal epoxidehydrolase (hmEH) plays a key role in the metabolism of the anti-epileptic drug carbamazepine. The main active metabolite formed by a not yet characterized cytochrome P450 enzyme is carbamazepine-10,11-epoxide. This epoxide is further hydrolysed by the hmEH to the carbamazepine-10,11-trans dihydrodiol which is o-glucuronidated and eliminated in the urine.
Different pharmacological studies showed that valpromide , another anti-epileptic drug prolongs the half live of carbamazepine-10,11-epoxide and that this effect is due to the inhibition of the hmEH.
We functionally expressed hmEH in *S.cerevisiae* yHE2 and showed that microsomes from this yeast strain give results comparable to other in-vitro studies concerning the inhibition of carbamazepine-10,11-epoxide hydrolysis by therapeutic concentration of valpromide.
The expression of hmEH in yeast offers a new in-vitro system to study the metabolism and drug interaction of possible substrates and inhibitors.

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DIFFERENTIAL ETHANOL CONSUMPTION IN ROMAN HIGH- AND LOW-AVOIDANCE (RHA AND RLA) RATS, BODY WEIGHT, FOOD INTAKE, AND THE INFLUENCE OF PRE- AND POST-NATAL EXPOSURE TO NICOTINE AND/OR INJECTION STRESS

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Male and female, 3 mo old RHA and RLA rats were given progressively increasing amounts of ethanol in their drinking water. RLA rats showed an aversion to drinking ethanol at doses above 2-4%, but RHA males and females increased consumption over controls, peaking at 6% and 8%, respectively. At those doses, the RHA rats were consuming about 60% more fluid than their RLA counterparts. Despite an overall, 30% reduction in food intake, the calories provided by ethanol maintained body weights at control levels, except in RLA males, which showed the strongest aversion to ethanol. Pre- and post-natal nicotine and physiological saline injections to the mothers of some of these RHA and RLA rats (3-4 times/day, nicotine=1.1-1.2 mg/kg/day) permanently, and selectively, reduced body weight only in RHA offspring (both males and females).

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SENSITIVITY TO ACUTE ETHANOL IN ROMAN HIGH- AND LOW-AVOIDANCE (RHA AND RLA) RATS: EFFECTS OF SEX, AGE AND PRE- AND POST-NATAL EXPOSURE TO ETHANOL

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Sleeping times were measured in 2 and 5 mo old female, and 2 mo old male, RHA and RLA rats following i.p. injections of 2.5 or 3.0 g/kg ethanol. No significant differences were seen between the RHA and RLA male groups at either dose, but all of the female groups differed significantly, with the RLA rats and the older rats being more sensitive (i.e. longer sleeping times). Body temperature was also measured every 30 min in the older female rats and the ethanol-induced hypothermia was stronger in RLA rats at all doses. Finally, RHA males whose mothers drank only 10% ethanol before and during pregnancy, and for 14 days after giving birth,were less sensitive to injections of 3.0 g/kg ethanol than were controls. The results indicate that responses to ethanol are genetically controlled, and that they may be correlated with other traits.

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ARYLAMINE N-ACETYLTRANSFERASE (NAT) GENES AND PROTEINS IN THE RABBIT

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NAT is the target of a common genetic polymorphism of drug metabolism in man. Polymorphic N-acetylation is also observed in the rabbit, which serves as animal model for the human defect. We have characterized two NAT genes (rgnat-a/-b) in the rabbit and provide evidence that a deletion of rgnat-b accounts for the observed polymorphism. Molecular analysis of the genes revealed that they consist of small noncoding upstream exons and single large coding exons of 870bp. Both genes were functionally expressed in COS-1 cells. Although the derived protein sequences (NAT-A, NAT-B) differed by only 13 amino acids, they displayed markedly different kinetic characteristics. NAT-A preferentially metabolized simple arylamine substrates such as p-aminobenzoic acid, whereas NAT-B showed high affinities for substrates containing bulky side groups (sulfamethazine). The structural similarity of the two proteins offers an ideal model system to study structure-function relationships of NAT-A and NAT-B.

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N-ACETYLTRANSFERASE (NAT) AND DEACETYLASE (DAC) ACTIVITIES IN DIFFERENT TISSUES OF THE RABBIT

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In rabbit two NAT enzymes (A,B) contribute to the biotransformation of pharmacologically and toxicologically important drugs such as isoniazid, procainamide and 2-aminofluorene. The fate or toxicity of an acetylated compound, however, may be further determined by the action of microsomal DAC. To determine the relative contribution of the two reactions we have analysed NAT and DAC activities in liver as well as in various extrahepatic tissues using the substrates p-aminobenzoic acid (NAT-A), sulfamethazine (NAT-B), and 2-acetylaminofluorene (DAC). Whereas NAT-B was only found in liver and duodenum, NAT-A and DAC were present in all tissues. NAT-A activities were similar in various tissues. DAC, however, showed a more complex pattern: Several tissues (e. g. duodenum, kidney) displayed significantly higher activities than liver,adrenal glands or lung. Generally lower activities were observed in muscle tissues.

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ASSESSMENT OF POTENTIAL DRUG RESISTANCE PARAMETERS IN CANCER

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Development of cytostatic drug resistance is a major problem in cancer chemotherapy. A number of mechanisms are suspected to contribute to this phenomenon. We are measuring the relative activities of several potential drug resistance mechanisms in tumour biopsies compared to the corresponding normal tissue from the same patient:
- Multidrug resistance gene (mdr) expression as judged by P-Glycoprotein staining on Western plots.
- Glutathione (GSH) levels, GSH-Transferase and GSH-Peroxidase activities.
- The level of the DNA repair enzyme O^6-alkylguanine-DNA-alkyltransferase.

To date we have assessed 35 cases of colon and breast cancer. We have found that none, several or all of the measured parameters may be elevated in the tumour as compared to the normal tissue. These results and their possible significance will be discussed.

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INCREASED ALDRIN-EPOXIDASE ACTIVITY AND SENSITIVITY FOR SELECTED P-450 DEPENDENT PROMUTAGENS IN THE DROSOPHILA WING ASSAY AFTER CHROMOSOME SUBSTITUTION

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Drosophila melanogaster larvae and adults have been found to be useful genotoxicity test systems capable of detecting effects of promutagens requiring different activation pathways. In the Somatic Mutation And Recombination Test (SMART) larvae trans-heterozygous for two recessive wing markers on chromosome 3 are exposed to test compounds. Genotoxic effects are detected as spots on the adult wing surface. In order to improve the metabolic efficiency of our tester strains with respect to promutagen activation chromosomes 1 and 2 have been substituted by those of a strain with a constitutively increased P-450 metabolism controled by a locus on chromosome 2. The sensitivity for a serie of promutagens requiring metabolic activation was demonstrated to be increased in the the new strains (e.g. N-Nitrosopyrrolidine). Beside of these in vivo data a strong increase of the P-450 dependent aldrin epoxidase activity was found in microsomal fractions of the new strains. Work supported by Swiss Cancer League.

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CHRONIC EXPOSURE TO LYSOSOMOTROPIC DRUGS CHANGES PLASMA MEMBRANE LIPID COMPOSITION OF CULTURED CELLS

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Chronic exposure of different cultured cell-types to lysosomotropic drugs changed the cellular phospholipid(PL)-metabolism. This resulted in an increase in the PL-content and in changes in the PL-composition. Since alterations in PL-composition and cholesterol-content are of relevance for most functional properties of the plasma membrane, it was mandatory to study the drug effects in highly purified plasma membrane preparations. The isolation of plasma membrane was achieved by vesiculation of control and drug exposed cells with 25 mM formaldehyde and 2 mM dithiothreitol. Measurements of membrane markers (5'-nucleotidase, cholesterol, sphingomyelin) and of a lysosomal marker (ß-D-Glucosidase) proved the ability of this method to isolate pure and representative plasma membrane vesicles (PMV). After chronic exposure of three cultured cell-types to lysosomotropic drugs of different therapeutic classes, the PL-composition of isolated PMV were determined by onedimensional HPTLC of membrane PL extracts. Changes in the lipid composition were smaller in membranes than in whole cells. While in drug exposed cells total membrane PL- and cholesterol-contents were generally decreased, the changes in the membrane PL-pattern were cell- and drug-specific. These drug-induced alterations in the plasma membrane lipid composition are discussed with regard to earlier described drug effects on membrane fluidity and on ß-adrenoceptor densities of cultured cells.

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ELECTRONIC DETERMINANTS OF POLYMORPHIC DEBRISOQUINE/ SPARTEINE CYTOCHROME P-450 INHIBITION

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Computational techniques have been used to search for the electronic and structural determinants of substances which competitively inhibit the metabolism of bufuralol by human cytochrome P-450IId1. A rigid substrate has been used as a model to identify the probable active conformation of molecules with flexible structures; quantum chemical and force field methods have been used to calculate properties which might be reactivity indices for complex formation. Good correlation has been obtained between the observed (apparent) binding constants and the interaction volumes (IV) calculated for the protonated compounds with O- as a probe; this is improved by including proton affinities (PA) in the regression. The correlations suggest that binding strength increases with increasing IV and decreasing PA. In spite of the high structural diversity of the substances studied, these results suggest that the basic nitrogen and its covalent environment are of major influence in binding.

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EFFECTS OF MERCURIC CHLORIDE ($HgCl_2$) ON AN EPITHELIAL CELL LINE ($LLC-PK_1$) : AN ELECTROPHYSIOLOGICAL APPROACH

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Inorganic mercury is known to induce nephrotoxic acute renal failure. The epithelial cell line $LLC-PK_1$, with proximal tubular cell properties, grown at confluence on collagen coated filters was used to monitor short-term changes induced by $HgCl_2$ on the resistance, potential difference (PD) and short circuit current (I_{sc}). The experiments were performed using a modified Ussing chamber allowing continuous perfusion (200 ml/h) of medium into the apical and basolateral sides at 37°C. $HgCl_2$ was added in a medium containing [mM] : Na^+ 137, K^+ 5.5, Ca^{++} 1.8, Mg^{++} 0.8, Cl^- 121, HCO_3^- 25, glucose 25. Transepithelial measurements were made under voltage-clamp conditions. The $LLC-PK_1$ developped a transepithelial PD of -1.2 mV and a resistance of 120 $\Omega \cdot cm^2$. Apical exposure to $HgCl_2$ (1 µM) induced an almost immediate increase (60 µA) of the I_{sc}. In contrast a basolateral exposition decreased (25 µA) the I_{sc}. The simultaneous addition of 1 µM $HgCl_2$ on both sides of the epithelium induced a transient increase followed by a decrease of the I_{sc}. These effects were nearly completely reversed by subsequent addition of 2 mM dithiothreitol. Thus, application of electrophysiological techniques to cultured epithelial cells should allow to specify the early mechanisms of functional alterations induced by toxic agents.

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NUCLEOTIDE SEQUENCE OF TWO LDH-GENES FROM B. PSYCHRO-SACCHAROLYTICUS

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Beeing interested in the nucleotide sequence of the LDH-gene from B. psychrosaccharolyticus, in order to study homologies to LDH's of different bacilli, we found two genes encoding for LDH. One, LDH(P), of which the deduced amino acid sequence is identical to the wildtype enzyme and a second one, LDH(X), of which the deduced amino acid sequence is 86 % homologous to the wlidtype enzyme from B. psychrosaccharolyticus. The nucleotide sequences of LDH(P) and LDH(X) show a homology of 77 %. Both genes are expressed in E. coli and could be isolated as shown by enzyme activity tests and N-terminal amino acid sequence. However, LDH(X) is not expressed in B. psychrosaccharolyticus itself under the conditions chosen for oxygen induction of LDH, although DNA sequence analysis revealed putative promotor recognition sites and a ribosome binding site (Shine-Dalgarno Box) which are strongly homologous to the postulated bacillus σ promotor and ribosome binding sites of bacteria. The functional significance of the LDH(X) is not known.

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MUTANT LACTATE DEHYDROGENASES WITH NEW PROPERTIES

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In order to better understand the contribution of the B-helix to the stability and activity of the thermophilic LDH from *B. stearothermophilus* and of the mesophilic LDH from *B. megaterium*, mutant enzymes were constructed on the basis of both wild types in the region of the B-helix. The mutations consist of single to quadruple amino acid exchanges (amino acids from the mesophilic species were cloned into the thermophilic LDH and vice versa) and of one hybrid construction. The kinetic properties and the quaternary structure of these mutants were determined and compared with both wild type enzymes. It could be shown, that specific mutations in the B-helix can lead to different characteristics of the enzyme. The kinetic mechanism of the mesophilic LDH could be changed by means of 2 mutations at the positions 37 (Ala → Val) and 40 (Met → Leu) from sequential ordered to random. Without the activator fructose 1,6-diphosphate (FDP), this mutant is not active and exists as a monomer, contrary to the wild type, which is a dimer. In the presence of FDP the mutant is active and exists, as in the wild type, as a tetramer. The mutations in the B-helix of the thermophilic LDHs lead to changed affinities to FDP, although the mutations are not located in or near the FDP binding site.

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ALTERING THE THERMOSTABILITY OF LACTATE DEHYDROGENASE FROM BACILLUS STEAROTHERMOPHILUS

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There have been made three point mutants of the LDH from B. stearothermophilus which all lie within the B-helix at the N-terminus. This helix forms the contact site between the subunits of the tetrameric enzyme over the Q-axis and was postulated to be very important for the thermostability of the protein. The amino acid substitutions (I: AA 36 Y -> S; II: AA 37 V -> S; III: AA 38 F -> S) aim to transform the thermophilic wildtype to more mesophilic mutants.
The mutations were introduced by synthetic oligonucleotides. The mutated LDHs were isolated by affinity chromatography over an oxamate-Sepharose column.
The properties of the mutants I and II have not altered much compared to wildtype. Mutant III however is very interesting: Its thermostability determined as residual activity after incubation for 30' at various temperatures has decreased 10°C compared to wildtype (85°C). The K_m for Pyruvat (16.7 mM) is constant at various concentrations of the activator FDP. K_{cat} increases about the factor 30 at FDP saturation compared to nonsaturation and reaches the values of the wildtype at FDP saturation (240 s^{-1}).

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DICHLOROMETHANE DEHALOGENASE OF METHYLOBACTERIUM SP. DM4 IS A GLUTATHIONE S-TRANSFERASE

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Methylobacterium sp. DM4 is a pink-pigmented facultative methylotroph utilizing dichloromethane (DCM) as the sole source of carbon and energy. The first step in the utilization of DCM is catalyzed by DCM-dehalogenase (DCMD), a strongly inducible enzyme which converts DCM into formaldehyde and inorganic chloride with GSH as a cofactor. The genes for DCM-utilization have been isolated and a 2.8 kb subclone was sequenced by the dideoxy-method. It contained the 867 bp DCMD structural gene (*dcm*A), the *dcm*A promoter and the *dcm*A upstream region involved in the regulated expression of *dcm*A.
Alignment of the deduced DCMD amino acid sequence with amino acid sequences of eukaryotic glutathione S-transferases revealed three regions containing highly conserved amino acid residues and indicated that *dcm*A is a member of the glutathione S-transferase supergene family.

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FITNESS OF E.COLI K12 DEPENDS ON THE EXPRESSION OF TN5 SEQUENCES CARRIED ON PLASMIDS.

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The plasmid pRAB2 contains silent neo (Km^r) and ble ($Bleo^r$) genes from the transposon Tn5. Spontaneous mutations leading to their expression, eg. by insertion of an IS element, result in selectable Km^r $Bleo^r$ cells. During an attempt to estimate transposition rates in relation to growth conditions (exponential growth vs. phase of decline), the proportion of Km^r $Bleo^r$ relative to Km^s $Bleo^s$ bacteria increased under non-selective conditions as a function of time of incubation. Competition experiments revealed a slower death rate of Km^r $Bleo^r$ cells during phase of decline. We describe the strategy to identify the factors responsible for the increase of fitness, and we discuss the possible molecular mechanism(s) involved in the higher viability caused by the mutant plasmids.

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TRANSFORMATION OF PROTOPLASTS BY MICROINJECTION: AN DETAILED ANALYSIS OF EFFICIENCY AND INTEGRATION PATTERN.

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Transformation of higher plant cells is one of the most important techniques in plant molecular biology and plant improvement. Several methods have been developed (Agrobac= terium, direct gene transfer, electroporation, biolistics, microinjection, or others). In perticular microinjection, which is now established for several years, has no been characterised in respect to the efficiency, type of integra= tion and transmission to the offspring. The experiments reported here make use of tobacco protoplasts as a model to study these parameters. For this purpose a new protoplast culture system had to be developed: Thin layer alginate embedding allowed us to investigate and elucidate the role of many parameters which might affect the integration of foreign DNA after the injection process.

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ABNORMAL CHROMOSOMAL ARRANGEMENT IN HUMAN OOCYTES

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There are many contradictions about the frequency of chromosomal anomalies in unfertilized human oocytes gained from patients undergoing IVF programs. The overall frequency of chromosomal abnormalities varies between 4 and 57% according to different studies.
Chromosome preparations were made using a modified method of Tarkowsky [1] in order to minimize the degree of chromosome scatter. Cytogenetic investigations were performed on 54 unfertilized oocytes, 36 (i.e. 66.7%) had a normal haploid chromosomal complement. The frequency of unspecific numerical aberrations were 11.1% for hypohaploid, 13.0% for hyperhaploid and 3.7% for hyperdiploidy. The total rate of aneuploidy was 27.8%. Two chromosomal complements had structural rearrangements (3.7%). It is not known if this high number of chromosomal aberrations is also true in vivo, or if there is, for example, an influence of the treatment procedure.

Reference:
[1] A.K.Tarkowsky, Cytogenetics (1966), 2, 394-400.

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DNA ANALYSIS OF SWISS AND AMERICAN STRAINS OF BORRELIA BURGDORFERI

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Lyme disease is a tick-transmitted spirochetoses caused by the bacteria Borrelia burgdorferi. Although Lyme disease in North America has many features in common with the European forms of infection, it differs in frequency and severity of symptoms. Strains isolated from ticks from the USA and Switzerland were compared at the DNA level. The relationships were determined by: 1) RFLP's of chromosomal DNA; 2) Profiles and sequence homologies of (a) conventional supercoiled plasmids and (b) linear plasmids which are a unique feature of Borrelia species.
From the RFLP analysis strains can be grouped according to location (USA or Switzerland) although a few Swiss strains resemble the USA group. All the strains contain circular and linear plasmids and can be similarly grouped based on number and size of plasmid species and sequence homologies.

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DNA-FINGERPRINT IN PIGS

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DNA-fingerprinting is an useful technique to identify individuals (e.g. animals, humans) or inbred strains (e.g. mouse). This method also can be helpful to perform gene mapping or to look how a trait is segregating within a family. By adapting this technique to pigs we plan to pursue traits in different pig families, to which clinical disorders or certain quantitative traits are associated. It was possible to get DNA-fingerprint pattern using the M13-probe and the Jeffreys probes 33.15, 33.6 respectively, provided the following experimental steps were optimized: (i) isolation of high molecular weight DNA (>50 kB), (ii) the use of the appropriate restriction enzymes and (iii) the stringency of the hybridization conditions. Results will be presented showing that different pig races can be distinguished with this method.

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TRANSFORMATION OF MERISTEMATIC CELLS: THE MICROINJECTION APPROACH

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One major problem in genetic engineering of cereals is the lack of an efficient transformation technique. To overcome the difficulties with biological vectors and protoplast regeneration, recently physical approaches (biolistics and microinjection) were made to introduce DNA into meristematic cells. To investigate the open question whether meristematic cells can be transformed at all, we apply microinjection to zygotic proembryos and embryos of the model plant Arabidopsis thaliana. A culture system for zygotic proembryos (globular and early heartshape stages) from A. thaliana has been established. Following microinjection these embryos were regenerated to plantlets either directly or via callus under selective pressure. Developing embryos, calli and shoots will be analysed for expression and integration of the injected gene.

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THE OLIGOMERIC STRUCTURE OF MALATE SYNTHASE (SOYBEAN COTYLEDONS)

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Malate synthase (EC 4.1.3.2) is a specific enzyme of the glyoxylate cycle, and has generally been considered as a peripheral glyoxysomal membrane protein. Our biochemical data now suggest that malate synthase is a matrix protein. The characteristics of the oligomeric structures of the enzyme might have led to misinterpretation of its possible membrane association. It appears that malate synthase can form three different oligomers with identical subunits (Mr 64'000). An aggregated form is obtained in the presence of 5 mM $MgCl_2$ (at low buffer ionic strength), which coprecipites with glyoxysomal membranes. In contrast, malate synthase is solubilized as a dimer at high ionic strength buffer (in the presence of 5 mM $MgCl_2$ and 50 mM KCl). A third soluble form (octamer) is obtained in the absence of $MgCl_2$ (at low buffer ionic strength). The aggregation/disaggregation phenomena can be efficiently used in the purification procedure, and it is therefore now possible to isolate malate synthase as a soluble protein.

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PRIMARY STRUCTURE OF FERREDOXIN-THIOREDOXIN REDUCTASE FROM SPINACH CHLOROPLASTS.

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The ferredoxin-thioredoxin reductase (FTR) reduces chloroplast thioredoxins, involved in enzyme regulation, with electrons from ferredoxin. The native protein, with a MW of about 30'000, contains an Fe-S cluster and catalytically active Cys. The results from amino acid composition and N- and C-terminal sequences show that spinach FTR is composed of two unidentical subunits (MW: A=17'200, B=15'400) present in equal amounts. Subunit A has a N-terminus of variable size containing many Ser and Pro residues. Subunit B contains the active Cys residues. We have used specific reagents to locate the active site disulfide and the Fe-S cluster within the primary structure. Whereas the redox-active disulfide was found in subunit B the Fe-S cluster seems to be located between the two subunits. (Aided by grant from the Swiss National Science Foundation).

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ISOCITRATE LYASE FROM GERMINATING SOYBEAN COTYLEDONS: NOVEL PURIFICATION PROCEDURE AND ACTIVATION BY FATTY ACIDS AND LIPIDS

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Lipid catabolism and gluconeogenesis are connected in germinating oleagineous seeds by the glyoxylate cycle. Isocitrate lyase (EC 4.1.3.1) is a key enzyme of this metabolic pathway, and is located in glyoxysomes.
The enzyme from various sources has already been characterized, and appears to be regulated by metabolites from the gluconeogenesis pathway.
Isocitrate lyase from soybean cotyledons has already been purified from crude extracts as well as from isolated glyoxysomes, but the reported purification factors and recovery yields are low. It has now been observed that the enzyme has hydrophobic characteristics, and a purification procedure with includes an efficient hydrophobic chromatography step can therefore be proposed.
The hydrophobicity of the enzyme might also explain the activation effects of various fatty acids and lipids.

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TRANSFORMATION OF THE MOSS *PHYSCOMITRELLA PATENS*

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Protoplasts of the moss ***Physcomitrella patens*** were transformed using a PEG procedure and expression vectors coding for kanamycin and hygromycin resistance. Two classes of transformants were obtained. The first, about 1 % of the clones, was characterised by a normal pattern of growth, stability of the new character in the absence of the selective pressure, meiotic transmission of the resistance and mendelian segregation. These clones were therefore considered to be true transgenic mosses. The second class was characterised by alteration in growth, development and loss of the resistance in the absence of the antibiotic. The new phenotype was mitotically unstable, and was transmitted at very low frequencies through meiosis. Segregation analysis and molecular data will be presented. We shall also discuss the nature of the second class of transformants.

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NEW PHENOTYPE PATTERNS REGENERATED BY GENETIC INSTABILITIES IN *PORTULACA GRANDIFLORA* (HOOK)

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The locus C of *Portulaca grandiflora* is responsible of synthesis of betalain pigments in petals.
Instabilities alter gene expression and regenerate new phenotypes patterns with different intensities of flowers pigmentation. This finding suggests that the gene may be rendered genetically hypervariable as a consequence of transposable elements insertion and excision.
To study this problem, we have analysed the phenotypic variation arising from unstable c* of *Portulaca grandiflora* .

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REGULATION OF C3 VERSUS C4 METABOLISM AND CELL SPECIFICITY IN ZEA MAYS DURING DEVELOPMENT.

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Maize is characterized by a C4-type carbon fixation metabolism. This system implies the differential compartmentalization of its enzymes: CO_2 is primarily fixed into oxaloacetate in the mesophyll cells by phosphoenolpyruvate carboxylase(PEPC), carried to the neighbouring bundle sheath cells, released, and refixed by ribulose-1,5-biphosphate carboxylase(RuBPC) in the Calvin Cycle. Under normal light conditions the O_2-sensitive RuBPC only occurs in bundle sheath cells and is in this way compartmentalized away from O_2, whereas low light grown plants express RuBPC in both cell-types and thus display an overall C3 metabolism. In this state PEPC is not expressed. Likewise RuBPC is predominant in very young leaves. We are interested in the molecular mechanism of regulation of this C3 versus C4 metabolism during plant development(using reporter genes, in situ hybridization, etc). Currently we are focussing on three light regulated and cell-specifically expressed genes involved in this pathway(small subunit of RuBPC, PEPC, phytochrome). We have in a first approach analysed the expression of the maize C4 PEPC in a C3-type plant(tobacco).

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ARE HYDROXYCINNAMOYLPUTRESCINES CAUSALLY INVOLVED IN THE TUBERISATION PROCESS OF POTATO PLANTS ?

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The possible role of hydroxycinnamoylputrescines (HCPs) in the tuberisation of potato plants was studied using an *in vitro* system. Minitubers in shoot cultures were obtained by increasing the sucrose content of the Murashige-Skoog medium (without hormones) from 2 to 8 %, in the absence or presence of benzylaminopurine (BAP). Feruloylputrescine (FP) and caffeoylputrescine (CP) levels increased with tuberisation, but only when the medium contained BAP. Addition of inhibitors of phenylalanine ammonia-lyase (PAL) and of polyamine biosynthesis to the medium containing BAP reduced the levels of FP and CP to values lower than those in the absence of BAP, but had not effect on tuber formation. These results indicate that the increase of HCPs during tuber formation is not casually related to the tuberisation process.

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METABOLISM OF INDOL-3YL-ACETIC ACID BY WALL-BOUND ENZYMES FROM MAIZE ROOTS

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The metabolism of indol-3yl-acetic acid (IAA) by enzymes bound to maize root cell-walls is analysed by HPLC, and compared with IAA metabolism in root segments. A cell-wall fraction was prepared, and ionically-bound enzymes were released by $CaCl_2$. The enzyme preparation was tested for its ability to catalyse the metabolism of (5-^{3}H)IAA by 2 different reactions: peroxidase and IAA-oxidase. No significant degradation of IAA by the peroxidase reaction was observed. In contrast, the IAA-oxidase reaction yielded at least 4 radioactive metabolites. The chromatographic and fluorescence properties of one of the major metabolites suggest that it is indol-3yl-methanol. When (1-^{14}C)IAA was used, no radioactive metabolites were detected, indicating that IAA metabolism by the wall-bound enzymes involves decarboxylation of the side-chain. For comparison, IAA metabolism was tested by incubating root segments in either (^{3}H)IAA or (1-^{14}C)IAA. Several metabolites were formed, and the pattern was very similar with either substrate: IAA decarboxylation was therefore not significant. It is suggested that the wall-bound enzymes may play a part in IAA metabolism in damaged tissue, when IAA is released into the cell-wall.

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REGULATION OF ASSIMILATORY SULFATE REDUCTION BY GLUTATHIONE IN LEMNA MINOR L.

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Glutathione (γ-Glutamylcysteinylglycin), the major non-protein thiol of higher plants, is considered to be the most important form for the storage and transport of reduced sulfur. When it was fed to Lemna minor at 1 to 2.5mM in the nutrient solution together with sulfate there was a decrease in the uptake of sulfate and in the activity of adenosine 5'-phosphosulfate sulfotransferase, a key enzyme of assimilatory sulfate reduction. There was a parallel increase in the non-protein thiol content and in the amount of sulfate present in the plants. With a combination of glutathione (2.5mM) together with aspargin and glutamin (2mM each) the decrease in adenosine 5'-phosphosulfate sulfotransferase activity was much less pronounced than with glutathione alone. Taken together these results show that glutathione regulates the uptake and the assimilation of sulfate and indicate an interaction between sulfate and nitrate assimilation. The increase in the sulfate content together with the decrease in sulfate uptake demonstrates that part of the glutathione taken up was oxidized.

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POSSIBLE PRESENCE OF A 75 kDa HEAT SHOCK POLYPEPTIDE IN THE STROMA OF SPINACH CHLOROPLAST

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High temperature stress or heat shock induces dramatic changes in gene expression in a wide variety of prokaryotic and eukaryotic organisms. In plants (soybean seedling tissues) several heat shock proteins (hsp) characterized by a low (less than 30 kDa) or higher (65 to 85 kDa) Mr have been identified (Key et al., PNAS US 78, 3826-30, 1981). Recently, Vierling et al., PNAS US 83, 361-5, 1986) have demonstrated that in soybean, pea and corn, nuclear-coded hsp having a Mr of 21 and 27 kDa are transported into chloroplasts. We shall report on a 75 kDa polypeptide encountered in the spinach chloroplast stroma. The antibodies raised against this polypeptide crossreact with the stroma fraction of three different plant species (tobacco, lettuce, pea) as well as with one of the three hsp 70 forms found in bovine muscle. On the other hand, antibodies against a highly conserved region of mammalian hsp 70 s react with a pea stromal polypeptide equivalent to the 75 kDa spinach polypeptide. Some of the biochemical and immunological properties of the spinach 75 kDa polypeptide will be presented. Supported by NSF 31.26386.89.

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STRUCTURAL INVESTIGATIONS ON THE CHLOROSOME OF *CHLOROFLEXUS AURANTIACUS*, A GREEN NON-SULFUR BACTERIUM

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Chlorosomes, membrane-bound oblong bodies rich in bacteriochlorophyll and protein, contain a powerfull light-harvesting antenna, the B740 complex. The absorption maximum of this antenna between 715 and 750nm enables Green Sulfur- and Green Non-Sulfur Bacteria to make effective use of the biological window between the absorption ranges of eucaryotic organisms, cyanobacteria and purple bacteria.

A model of the B740-complex, based on EM-data, enzymatic cleavage experiments, protein/pigment relations and the primary structure of the main chlorosome protein, was created by Th.Wechsler et al. in 1985. In this model 12 subunits of BChlc-binding protein, -each of them associated with 7 BChlc-molecules-, constitute the B740 complex. Recent results of K.Griebenow and A.Holzwarth however using a gel-electrophoretic filtration method for the isolation of chlorosomes suggested the predominant existence of a protein-free B740 complex.

By our investigations we are now able to prove, that the BChlc-binding protein is located in great quantities in the interior of the chlorosome and so most probably takes part in the formation of the B740 complex. Further results provide new insight into the structure of the chlorosome.

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ORGANELLAR FATE UPON SOMATIC HYBRIDIZATION BY MICROFUSION OF DEFINED PROTOPLAST PAIRS IN NICOTIANA

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Somatic hybrid/cybrid plants produced by one-to-one electrofusion of defined selected protoplast-protoplast and protoplast-cytoplast pairs (**microfusion**) of male sterile N. tabacum and cms N. tabacum (N. bigelovii) lines and **microculture** of the fusion products were analyzed for their organellar composition.
The fate of chloroplasts was assessed by a streptomycin resistance/sensitivity (trait encoded by the chloroplast genome) assay using somatic leaf tissue (R_0 generation) and R_1 seedlings.
For the analysis of mitochondrial (mt) DNA, species specific patterns were generated by Southern hybridization of restriction endonuclease digests of mtDNA and total DNA with three DNA probes of N. sylvestris mitochondrial origin.
In addition, variation in flower morphology, developmental histology and SEM studies on floral bud formation were analyzed within independent regenerants from the same fusant and between different independent fusion products. In this way, transmission genetic analysis concerning organelle segregation patterns as well as effects of nuclear-cytoplasmic interactions on flower ontogeny in somatic hybrids/cybrids obtained by microfusion of defined preselected protoplasts is reported for the first time.

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CELL SURFACES AND GLYCOPROTEINS IN THE INTERACTION BETWEEN PHYTOPHTHORA MEGASPERMA AND ITS HOST PLANT, GLYCINE MAX (L. MERR.)

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Specific contact between components from the cell surfaces of fungal pathogen and plant host may represent a main recognition event in these host-pathogen systems. Mechanically isolated mesophyll cells of soybean and germinating cysts of Phytophthora megasperma were cocultured and the interactions studied at the light microscope level. Reactions observed included the formation of wall appositions (callose) by the host and, sometimes, the formation of appressoria. Using lectins as probes, the presence of glucose and mannose residues could be demonstrated on the surface of soybean cells and of germinated cysts. Furthermore, galactosyl and fucosyl receptors were shown to be present on the latter. Monoclonal antibodies against surface (glyco)proteins, from germinated cysts and from leaves (intercellular washing fluids) of non-infected and infected leaves of soybean, were produced. Immunoblots showed reactivity against different glycoproteins sharing a common carbohydrate epitope most likely involving a N-Acetyl-D-glucosamine and lactose residue.

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NEW SYNTHETIZED STRESS-PROTEINS AND THEIR LOCATION IN MAIZE ROOTS

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Radioisotopic methods, electrophoresis and fluorography have been used to study biosynthesis of stress proteins in primary roots of Zea mays L. LG 11 seedlings. Stress as heat shock induced formation of few new proteins (heat shock proteins: HSP), as described by many investigators. Using linear sucrose gradients, we have located them in soluble extracts fractions and in different cell membranes. Results of experiments done at different temperatures showed that there is a threshold at 40°C for the synthesis induction. The temperature increase between the normal- and the shock-growth state seems to be not determinant. Other stresses (chilling, osmotic shock, ...) were assayed to test the root response and the possible formation of new, perhaps identical proteins.
Experiments with inhibitors of the RNA biosynthesis were performed to determine if new RNA formed was required for the HSP induction.

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PROTEIN CATABOLISM IN ISOLATED PEA CHLOROPLASTS

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Endopeptidases and aminopeptidases have been identified in chloroplasts of various plant species, but the control of proteolysis in plastids is not yet clear. Regulatory effects of solutes on the catabolism of chloroplast proteins were the focus of this work. Intact chloroplasts were isolated from pea (Pisum sativum L.) on Percoll steps (21, 60 and 80%) and incubated with different metabolites in the dark at 25°C. Since some chloroplasts burst during incubation, a second isolation of intact organelles was necessary prior to detecting the remaining polypeptides by SDS-PAGE and Western blotting. RubisCO protein was gradually degraded within 40 h and a 37 kD-polypeptide (reacting with antibodies against RubisCO) accumulated. The degradation of RubisCO and the accumulation of the 37 kD-polypeptide was delayed in chloroplasts after addition of inorganic phosphate, phosphoglyceric acid, dihydroxyacetone phosphate or oxalacetate. Other chloroplast proteins (glutamine synthetase, phosphoribulokinase, major thylakoid polypeptides) were affected differently by these treatments. The exchange of metabolites between the cytosol and the chloroplasts might be important for the control of catabolic processes in these organelles.

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PURIFICATION OF CHORISMATE SYNTHASE FROM A CORYDALIS SEMPERVIRENS CELL CULTURE

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Chorismate synthase catalyzes the last step common to the biosynthesis of the three aromatic amino acids in the shikimic acid pathway. The enzyme was purified from acetone powder extracts almost 1000-fold to near homogeneity from a cell culture of Corydalis sempervirens with 13 % yield. This is the first report on the purification of chorismate synthase from a higher plant source. Purification included chromatography on DEAE-cellulose, hydroxylapatite, cellulose phosphate and blue dextran-agarose. The preparation showed some minor impurities on silver stained polyacrylamide gels, which are likely to be degradation products. The subunit molecular weight is 41.9 kDa, that of the native enzyme 80.1 kDa, judged by SDS-PAGE and gel filtration, respectively. Antisera directed against the 41.9 kDa protein also detected the native enzyme. The identity of the purified protein was further confirmed by sequence analysis of tryptic peptides which showed homology with known sequences of the E. coli and N. crassa chorismate synthases.

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Betalain Biosynthesis in Beta vulgaris Cell Suspension Cultures

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The betalains are naturally occurring yellow (betaxanthins) and violet (betacyanins) pigments in plants of the order Centrospermae. *B. vulgaris* cell lines capable of synthesisng either betaxanthins, betacyanins or betaxanthins + betacyanins have been developed. The work presented in this poster will detail growth and pigment kinetic data for *B. vulgaris* cell suspensions maintained in shake flasks and a 13 litre air-lift bioreactor. The development of batch or continuous plant cell cultures in bioreactors is studied with the prospect of a possible industrial production of yellow and red pure plant pigments. It has been proposed that in view of recently highlighted health and safety problems with certain synthetic food colourants and the general public feeling toward artificial food additives that the betalains may be suitable as natural replacements for some food colourants in current use.

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CAPILLARY GROWTH IN THE CHICKEN CHORIO-ALLANTOIC MEMBRANE: A MODEL FOR THE INTUSSUSCEPTIVE MICROVASCULAR GROWTH?

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In rat lungs evidence was provided for a new type of microvascular growth called intussusceptive capillary growth of the microvasculature (Caduff et al., 1986). According to this concept new capillary meshes are added by formation of slender (Ø ~ 1 µm) transcapillary tissue pillars and not by sprouting of endothelial tubes. In search for an in vivo model for dynamic studies, we found the chorioallantoic membrane (CAM) capillaries of chicken embryos cultivated in Petri dishes to be a well suited system: In vivo microscopy combined with video-monitoring was possible for up to twelve hours. The CAM-capillaries resembled closely the pulmonary microvasculature with numerous slender pillar-like capillary meshes. Within hours we observed a massive and often excentric growth of the pillars, a shifting of their positions and sometimes confluence of smaller pillars to a single one. Contraction and dilatation of the observed CAM area were consequences of these changes. These dynamic changes suggest that the CAM-capillary network may also expand by intussusceptive growth.

Caduff et al., Anat.Rec.216,154-164.1986

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INFLUENCE OF POSTNATALLY ADMINISTERED GLUCOCORTICOIDS ON RAT LUNG GROWTH

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Daily glucocorticoid (GC) treatment from postnatal day 4 to 13 accelerated alveolar wall thinning and prevented alveolar formation (Massaro and co-workers, 1985). A prerequisite for interalveolar septation is the presence of a double capillary network (Burri 1985). Based on the hypothesis that GC prevented alveolar formation indirectly by inducing a premature microvascular maturation, we investigated qualitatively and quantitatively the ultrastructural changes of the capillary bed under GC-treatment. In GC-rats thinner septa were found on days 4 and 7. From day 7 onward septa with a single capillary system were found with increasing frequency in GC-treated rats, so that up to 80% of the septa were mature on day 10 (compared with only ~ 50% in controls). EM-morphometry revealed a significantly lower interstitial tissue mass in GC-rats on days 10 and 13. These preliminary results support the hypothesis that a precocious maturation of the capillary bed could be at the origin of the reduced alveolization.

Massaro et al., J. Clin. Invest. 76, 1297-1305, 1985
Burri,P.H.,Handbk. Physiol. Sect.3, Resp. Syst. 1-46,1985

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ACCUMULATION OF MITOCHONDRIAL CREATINE KINASE (MiCK) AT PARACRYSTALLINE INCLUSIONS IN MITOCHONDRIA OF ADULT RAT CARDIOMYOCYTES

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Adult rat cardiomyocytes cultured in essentially creatine-free medium for ten days show a varying number of elongated and enlarged mitochondria preferentially arranged in a perinuclear fashion as judged by confocal microscopy (Eppenberger et al., 1989). When exposed to monoclonal anti-MiCK antibody followed by rhodamine-conjugated second antibody, a stronger fluorescence could be observed within these elongated mitochondria as compared to the small round mitochondria also present in the cells. TME of high-pressure frozen and freeze-substituted cardiomyocytes embedded in Epon showed paracrystalline inclusions within the elongated mitochondria, similar to mitochondria of skeletal muscle under ischemia or after feeding animals with the creatine analogue GPA (Hanzlikova & Schiaffino, J. Ultrastruct. Res. 60, 121, 1977; Ohira et al., Jap. J. Physiol. 38, 159, 1988). Postembedding immunogold labelling using polyclonal anti-MiCK antibodies revealed a specific localization of MiCK within the paracrystalline structures suggesting to be one of the major components. These results may point to a compensatory accumulation of MiCK at the mitochondrial inclusions as a consequence of metabolic stress (creatine deficiency). Supported by SNF grant No. 3.497.86.

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DEGENERATIVE AND REGENERATIVE MUSCLE CHANGES IN HUMANS AFTER A HIGH ALTITUDE SOJOURN AT >5000M.

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Muscle ultrastructural changes during a typical expedition to the Himalayas were analyzed morphometrically for mitochondrial, myofibrillar, lipofuscin, satellite cell and myonuclei volume densities by taking muscle biopsies from seven climbers before and after their sojourn at high altitude (>5000m for over 8 weeks). Computer tomographs of the thigh were also taken to assess changes in muscle cross-sectional area. A significant reduction (-10%) of muscle cross-sectional area was observed as a result of a decrease in muscle fiber size due to a loss of myofibrillar proteins and a reduction in mitochondrial protein volume. A significant increase of the volume density of lipofuscin (+330%) was observed. The lipofuscin mostly appeared in the subsarcolemmal region near the poles of myonuclei. A significant increase of the volume density of satellite cells (+290%) was also found. This increase was due to similar increases of both the cytoplasm and nuclear volume of the satellite cells. The volume density of myonuclei showed no change with high altitude exposure. The considerable increase in lipofuscin seems to be related to muscle atrophy, while the increase in satellite cell volume is indicative of fibre regeneration.

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α2,3- AND α2,6-LINKED SIALIC ACID LOCALISED IN COLON AND KIDNEY USING MAACKIA AMURENSIS AND SAMBUCUS NIGRA LECTINS

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Maackia amurensis leukoagglutinin (MAL) and Sambucus nigra L. agglutinin (SNA) are known to react with sialic acid present in specific linkages; Neu5Ac(α2,3)Gal and Neu5Ac(α2,6)Gal sequences, respectively. We analysed the distribution of these two linkages. MAL complexed with 8 nm gold particles gave specific stainings in paraffin, Epon and Lowicryl K4M sections. Specificity was established by preincubation with α2,3 sialyllactose. We observed staining over the mucus of colonic goblet cells which was sensitive to β-elimination and resistant to N-glycanase treatment. Thus the MAL also recognizes sialic acid residues present on O-glycosidically linked oligosaccharides. The two lectines gave different staining patterns in rat kidney. The MAL-gold stained all capillaries, thick ascending limbs and collecting ducts without principal cells but not visceral glomerular epithelium, S1 segment of proximal tubules or urothelium, while the SNA-gold stained all structures. MAL-gold complexes can be used for the cytochemical detection of the Neu5Ac(α2,3)Gal sequence. Combination of MAL- and SNA-gold complexes enable the differential detection of the two types of sialic acid linkage on histological sections.

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N-CAM POLYSIALIC ACID LOCALISED IN EMBRYONIC RAT KIDNEY

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The distribution of polysialic acid (PSA) and N-CAM was investigated in rat embryonic kidney (metanephros). Tissue sections were immunostained using a gold-labelled mAb which recognised only long chain ("embryonic") PSA. PSA and N-CAM staining was generally coincident. At embryonic day 15 (E15), branches of the uretic bud and the condensed intermediate mesoderm, representing derivatives of the two embryonic anlagen involved in kidney formation, were PSA reactive. The first nephrons were initiated at E16. By E18, collecting ducts, cells of the presumptive Bowmans capsule and well developed glomeruli were unstained. Cortical collecting tubules stained weakly, while derivatives of the intermediate mesoderm *ie;* undifferentiated mesenchyme, primitive tubules, neprogenic condensations and cells of the S-shaped stage of nephron development stained strongly. During the nephrogenic conversion of mesenchyme to epithelium, baso-lateral sorting of N-CAM and PSA was seen in forming epithelia by immuno-electron microscopy. Derivatives of the two embryonic anlagen of kidney thus showed independent, developmentally regulated, N-CAM PSA expression patterns.

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CLEARANCE OF PARTICLES FROM INTRAPULMONARY AIRWAYS IN HAMSTERS IS NOT COMPLETED WITHIN 24 HOURS

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To test the generally accepted hypothesis that inhaled insoluble particles are cleared completely from the conducting airways within 24 hours we investigated 1) quantitatively the particle retention in intrapulmonary airways of hamsters immediately and 24 hours after inhalation and 2) the structural association of these particles. Seven hamsters inhaled an aerosol of 6μm particles. The lungs of 3 animals were fixed *in situ* 20 min after the end of the inhalation whereas those of 4 animals were fixed 24 hours later. The number of particles was estimated stereologically by the fractionator and the location was determined by light and electron microscopy. From the particles retained in the intrapulmonary airways immediately after inhalation about 15% were still retained 24 hours later. They were found in close contact with the epithelial cells. More than 90% were found to be phagocytized by macrophages.

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IMMUNOCYTOCHEMISTRY SHOWS BASOLATERAL ACCUMULATION OF THE CANALICULAR BILE SALT TRANSPORTIN PROTEIN (cBSTP) FOLLOWING BILE BUCT LIGATION IN RAT HAPATOCYTE PLASMA MAMBRANES

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Extrahepatic obstructive cholestasis induced an accumulation of functional cBSTP in the basolateral domain of isolated plasma membranes (JCI 84:876,'89). In order to further characterize this reversal of bile secretory polarity we examined the effects of bile duct ligation (50h) on the distribution of cBSTP in the three domains by electron microscopy using a polyclonal antibody (JBC 262:11324, 1987). K4M sections were immunolabeled with 5 nm colloidal gold and label was quantitated stereologically. Normal livers showed intensive labeling of the canalicular domain but very weak reactivity of the basolateral membrane. Bile duct ligation decreased number of immunogold particles by 69% in the canalicular membrane, and increased it by 13% in the basolateral domain. This shift is in line with our earlier functional and biochemical data.

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BILE DUCT LIGATION (BDL) INDUCES DE NOVO FORMATION OF CANALICULI AND BILE DUCTS IN RAT LIVER.

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In order to quantitate structural alterations following obstructive cholestasis we performed a stereological study on rat livers subjected to BDL for 2 days (n=5, sham operated controls n=5). After perfusion fixation thin sections and freeze fracture replicas were examined. BLD induced an increase in volume density (Vv) of portal fields (+ 167%) at the expense of parenchyma (-5%). Bile ducts increased in length (+70%) and number of cholangiocytes (+219%) while hepatocytes decreased in Vv (-8%) and increased in number (+9%). Canaliculi increased in length (+72%), volume fraction (+219%) and surface density (+63%). Alterations of tight junctions included a decrease in strand number (4.5 vs 2.9), junctional depth (250 vs 170 nm) and total strand length (5.5 vs 4.4 cm/cm canaliculus). Our data indicate de novo formation of canaliculi and bile ducts and are compatible with an increased paracellular permeability.

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THE MORPHOLOGY OF PRESUMED TENSION RECEPTORS IN THE DIGESTIVE TRACT OF THE RAT

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Gastrointestinal tension receptors as described in physiological studies are commonly assumed to be located in the muscular wall (1). Their structure, however, has not yet been elucidated. Using anterograde tracing techniques, two types of afferent fibers could be defined morphologically. The first type was found arborizing in the inner zone of the circular muscle layer parallel to the muscle fibers. it is abundant in spincters (pyloric ans internal anal) and originates from both nodose and spinal ganglia. The second type formed dense baskets around myenteric ganglia in the esophagus, stomach and small intestine, but not in the rectum and originates in nodose ganglia. The ultrastructure of these latter terminals suggests both a mechanosensory and also a local effector function related to enteric neurons. (Supported by SNF grants 3.555-0.86 and 31.25710-88)

1) PLR Andrews, Progr. Brain Res. 67(1986)65-86

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NON-INVASIVE EVALUATION OF AUTONOMIC NERVOUS SYSTEM ACTIVITY BY HEART RATE SPECTRAL ANALYSIS DURING TILT IN HUMANS

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R-R intervals are measured on continuous ECG by electronical R detection. Series of intervals are computerized off-line to provide power spectra using FFT algorithms. Respiratory movements (at fixed frequency) and finger arterial pressure are also continuously recorded. The low frequency (LF) peak (0.02 Hz to the lowest respiratory frequency limit) of spontaneous heart rate fluctuations are known to be linked to both parasympathetic and sympathetic nervous systems, and the respiratory frequency peak (RF) (around 0.25 Hz) to be of parasympathetic origin only. The results obtained on 12 subjects who performed 60 degrees head-up-tilt are presented. A decrease of the RF, and an increase of the LF and of the LF/RF ratio during the tilt were observed, in agreement with other studies. Furthermore an index of peripheral resistance was calculated from the blood pressure decrease by fitting it with an exponential function. Increase in resistance is concomitant with increasing LF/RF ratio, i.e. changes in sympatho-parasympathetic balance toward sympathetic. Since the precision of the electronical R detection allows to take only 64 or 128 intervals per series, a spectrum can be taken every 1 or 2 minutes.

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SIMILARITY OF THREE MODELS WHEN ESTIMATING OXYGEN DIFFUSIVE CONDUCTANCE IN MUSCLE TISSUES

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Three mathematical approaches for estimating the blood to mitochondria diffusive conductance for oxygen, $GdiffO_2$, in O_2 supply limited muscles have been studied. They correspond to three capillary-tissue models : a) the inversed alveolar-capillary model, b) the centrally perfused Krogh cylinder model and c) the peripherally perfused cylinder model of Hill. In all three models mean capillary PO_2 was computed and mean tissue PO_2 was either assumed (model a) or computed (models b and c). We demonstrated that $GdiffO_2$ estimates made at the critical O_2 supply point are most sensitive to model choice, the Hill model yielding the highest and the inversed alveolar-capillary model the lowest estimate. However, analysing data obtained from muscles working with O_2 supply well below critical reduced the difference in $GdiffO_2$ estimates among models, until, under very critical conditions, all 3 models converge. Thus our data suggest that in circumstances where VO_2max is likely to be diffusion limited, all three models can be interchangeably used to compute estimates of tissular $GdiffO_2$.

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MEASURING HUMAN MUSCLE GLYCOGEN WITH 13-C NMR SPECTROSCOPY IN VIVO

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Glycogen is the major storage compound of carbohydrates in muscle and reduced glycogen levels may limit performance. Conventionally, the glycogen content of human muscle has been determined by means of needle biopsies. 13-C NMR spectroscopy has been introduced as an alternative (T. Jue *et al.*, Proc. Natl. Acad. Sci. 86: 1439, 1989) which is non-invasive, can be repeated for kinetic studies of glycogen metabolism and yields results immediatly. Technical difficulties arrise because NMR has a low intrinsic sensitivity and the natural abundance of 13-C is only 1%. Although the total amount of glycogen in muscle is 70 - 110*mmol/kg* wet weight, the low effective sensitivity of detection requires special NMR techniques. We used numerically optimized adiabatic pulses and single frequency 1-H decoupling with a 5.5*cm*, double tuned surface coil and optimized the acquisition parameters and the examination protocol to observe the C-1 position of muscle glycogen at natural 13-C abundance. The feasibility of following changes in muscle glycogen levels was established in calf muscle of healthy volunteers before and after long distance running.

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ANISOTROPY OF MITOCHONDRIAL MEMBRANES IN MUSCLE FIBERS OF THE CAT

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We studied the question whether muscle mitochondria and their membrane systems show a spatial orientation with regard to muscle fibers. This was studied in oxidative M. soleus and glycolytic M. gracilis of the cat. Muscle tissue was processed for electron microscopy and a test system consisting of horizontal and vertical lines was aligned with the myofibrils on longitudinal sections. It was found that significantly fewer intersections with mitochondrial, outer membranes were found with lines arranged parallel to the major fiber axis (ratio parallel/cross 0.8 ± 0.02 $p<0.01$). In contrast, intersections with cristae showed similar numbers of intersections with both counting directions. These results indicate that because of the outer mitochondrial membrane anisotropy, unbiased estimators of this variable must be obtained by using longitudinal (vertical) sections and a cycloid test system according to Mattfeldt (1985). (Supported by grants from Swiss NSF).

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DISCHARGE PATTERNS OF PULMONARY RAPIDLY ADAPTING STRETCH RECEPTORS (RAR) DURING HIGH-FREQUENCY OSCILLATORY VENTILATION (HFOV).

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RAR respond by increased discharge rate to lung deflation, large inflation as well as to inhalation of chemical irritants. RAR are known to prolong inspiration, shorten expiration and elicit rapid and shallow breathing. The question arises, if RAR are stimulated by HFOV, as the respiratory response to HFOV also involves elements resembling the respiratory effects mediated by RAR. Therefore, HFOV-induced changes of RAR activity were analyzed in anaesthetized rabbits by means of single fibre preparations of RAR afferents. Surprisingly, a wide spectrum of RAR discharge patterns during HFOV was found. RAR activty was either increased or decreased or unchanged during HFOV. The majority of 60 afferents examined showed either positive or negative correlation with airway pressure. It is concluded that the heterogeneous changes of RAR activity may explain the variability of respiratory responses to HFOV in dependence on HFOV-parameters used.

Supported by the Swiss National Foundation, grant Nr. 32-9045.86 and by Roche Research Foundation.

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ACUTE ALTITUDE EXPOSURE OF ACCLIMATIZED AND NON-ACCLIMATIZED SUBJECTS

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In order to compare the respiratory, cardiovascular, renal and psychomotor reactions to acute hypoxia of acclimatized climbers returning from a Himalaya-expedition and of non-acclimatized volunteers (students), the subjects underwent a standardized stepwise ascent to 6000m (PAO2=35mmHg) in a low pressure chamber. The duration of the entire altitude exposure, apart from the adaptation time before ascent, was 2h. The results show the following significant differences between the two groups: The acclimatized subjects exhibited a lower but more efficient hyperventilation, a lower (calculated) cardiac output, neither diuresis, nor natriuresis, no depression of central vasomotor tone and no abatement of psychomotor function (attentiveness), this in contrast to the control group. It is concluded that acclimatized climbers in contrast to non-acclimatized subjects resist hypoxic hypoxia better by economizing ventilatory and cardiovascular reactions.

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Effect of Na-salicylate on lung diffusing capacity

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Oxygen binding and release from hemoglobin is slower when hemoglobin is enclosed in the red cells (RBCs) compared to an equivalent hemoglobin solution. In-vitro experiments, using rapid reaction techniques, had identified an extraerythocytic diffusion obstacle, the so-called unstirred layer (USL) as the cause of this difference. The present study was undertaken with the premise that USL contributes measurably to the transfer resistance of oxygen in the lung. The hypothesis to be tested was that the convective mixing around the RBC could be increased through increased deformability of the RBC, thus diminishing the obstacle induced by USL. The increase in deformability was induced by Na-salicylate. Experiment: Isolated, ventilated rabbit lungs were perfused with washed human RBCs in Ringer+Dextran. After control measurements of gas exchange parameters, Na-salicylate was added (approx. 1.5 to 3 g/l of perfusate). Lung diffusing capacity for oxygen increased from 0.45 to 0.54 ml/min/mm Hg, i.e. to 121 ± 4 % (mean ± SEM, n=10) of the control value. Although this increase cannot be ascribed with certitude to the flexibilizing effect Na-salicylate, the results of a control study do not exclude this possibility. Indeed, when perfusing the same preparation with hemoglobin solution, no effect of Na-salicylate could be observed. Diffusing capacity under Na-salicylate was 101 ± 6 % (SEM, n=7)of the control value.

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NON - INVASIVE MEASUREMENT OF REGIONAL CEREBRAL BLOOD FLOW IN RATS BY MAGNETIC RESONANCE IMAGING

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During i.v. infusion of Gd(DTPA), a paramagnetic contrast agent not crossing the BBB, the reduction of the local signal intensity in T2 weighted MR images of the brain should depend on the regional cerebral blood flow (rCBF). In normal rats, the effects of Gd(DTPA) during the first min of infusion varied regionally, corresponding to the known pattern of rCBF. The signal attenuation was e.g. big in the cortex and small in the corpus callosum. In a rat model of embolic stroke (permanent left middle cerebral artery occlusion pMCAO), the brain regions at risk of becoming necrotic (i.e. ipsilateral cortex and striatum), which are detectable by MRI (due to edema) only 6 h after pMCAO, are visible instantaneously using this method, due to changes in perfusion. A good correlation with a standard method for rCBF measurement (C-14-iodoantipyrine) suggests that this new, non-invasive method can be used to quantitatively determine rCBF in small laboratory animals.

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COMPUTER SIMULATION PROGRAM FOR TESTING SOFTWARE USED IN AUTOMATIC RESPIRATION ANALYSIS

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Measurement of respiratory parameters during acute altitude exposure of humans in a low-pressure chamber requires an automatic breath-by-breath analysis. Recently, we developed a new improved software which synchronizes respiratory gas flow and gas concentrations and in addition handles more than fifty other parameters.
In tests with volunteers or with a mechanical lung model it is hardly possible to differentiate errors arising in the measurement system (e.g. delay of gas concentrations, response time of apparatus) from those in the new software itself. Therefore, we developed a computer program which simulates the single calibration procedures and the single respiratory parameters (flow and respiratory gases given by a theoretical lung model) as well as the whole measurement system with its inadequacies.
The advantages of this procedure are (1) a safe check of the new software with its modifications and (2) the quantification of errors caused by the measurement inadequacies.

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SEASONAL CHANGES IN 24 H AND BASAL ENERGY EXPENDITURES IN RURAL GAMBIAN MEN MEASURED IN A RESPIRATION CHAMBER

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Adaptation of energy expenditure to seasonal variations in food availability was studied using a respiration chamber in a rural village of The Gambia (West Africa) in 18 Gambian men on 3 occasions : 1) at the end of the rainy season characterized by low food availability, 2) during the favorable dry season, and 3) at the onset of the following rainy season. From period 1 to 2, body weight increased by 2.8±0.4 kg; this was accompanied by a rise in 24 h energy expenditure from 2054±51 to 2191±54 kcal/d ($p<0.001$). From period 2 to 3, the mean body weight remained stable whereas 24 h EE decreased to 2089±46 kcal/d, a value similar to that observed in period 1.

Dietary-induced thermogenesis calculated for 3 meals increased significantly from period 1 to period 2 : 5.9±0.5 to 8.2±0.8% ($p<0.05$) and subsequently decreased during the rainy season to 3.6±0.6% ($p<0.001$ period 1 vs 3).

It is concluded that in rural Gambian men, metabolic adaptations in response to seasonal changes in food availability are reflected by a decrease in body weight which mainly consists in a loss of fat-free mass, accompanied by a decreased 24 h EE and a lowered dietary-induced thermogenesis.

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INCREASED THERMAL INSULATION DURING THE LUTEAL PHASE OF THE MENSTRUAL CYCLE IN WOMEN

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In order to study the influence of the menstrual cycle on whole-body thermal balance and on thermoregulatory mechanisms, metabolic heat production (M) was measured by indirect calorimetry and total heat losses (H) by direct calorimetry in 9 non-obese women during the folicular (F) and the luteal (L) phases of the menstrual cycle, while they were exposed for 90 minutes to neutral environmental conditions in a direct calorimeter. The menstrual cycle had no effect on M and H. In both cases M was similar to H (69.7±1.8 and 72.1±1.8 W in F and 70.4±1.9 and 71.4±1.7 W in L phases respectively indicating that the women were in thermal equilibrium. Tympanic temperature (T_{ty}) was 0.24±0.07°C higher in L than in F phases ($p<0.05$), whereas mean skin temperature (T_{sk}) was unchanged. Calculated skin thermal insulation was higher in the L phase (0.056 ± 0.002°C.m^2 W^{-1}) than in the F phase (0.050 ± 0.03°C.m^2.W^{-1}, $p<0.05$). Calculated skin blood flow was lower in the L phase (0.173 ± 0.008 1.min^{-1}.m^{-2}) than in the F phase (0.204 ± 0.015 1.min^{-1}.m^{-2}) ($p<0.05$). It is concluded that during the L phase, an increased thermal insulation of women exposed to a neutral environment allows the maintenance of a higher internal temperature than in the F phase.

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COMPETITION FOR SUCROSE-PELLETS IN RATS: EFFECTS OF CAFFEINE, THEOPHYLLINE AND SOME ADENOSINE ANALOGS.

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Within triads of male Wistar rats rank-orders can be discerned based on each individual's competition score for sucrose-pellets(1). We here describe the effects of caffeine(CAF), theophylline (THEO) and some adenosine-agonists on either poor-performing rats (which abstain from competition) or high-performing rats (which win competition). CAF and THEO temporarily helped poor-performing rats to overcome their abstention from competition. 8-sulfonyl-theophylline (S-theo, which poorly penetrates into the brain) was less effective . NECA (A2-agonist), as compared to CPA or CHA (A1-agonists), more potently inhibited the high-performing rats'competition. Obviously, adenosinergic drugs can affect competition-rates, too. The present data hint at a central action (CAF, THEO > S-THEO) and at an A2-mechanism (NECA > CPA, CHA). With view to the neurochemical unspecificity of CAF and THEO our findings will be compared with other, previously reported pharmacological manipulations.

(1) Gentsch et al. Behav.Brain Res. 27:37 (1988)

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FACIAL MOTONEURONES IN THE NEWBORN RAT ARE SENSITIVE TO VASOPRESSIN.

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The facial nucleus of the newborn rat contains specific, high affinity binding sites for arginine vasopressin. The presence of these sites is transient : their density decreases following postnatal day 13 and they are no longer detected in the adult rat. Thus, early in ontogenesis, facial motoneurones may be sensitive to vasopressin. We therefore carried out single-unit extracellular recordings from the facial nucleus in brainstem slices of the newborn rat. Thirty-three out of 40 neurones were excited in a reversible, concentration dependent manner by vasopressin at 5 to 1000 nM. Oxytocin had a much weaker effect. The action of vasopressin was blocked by an antivasopressor antagonist and was mimicked by a vasopressor agonist, [Phe^2,Orn^8]VT. By contrast, the selective antidiuretic agonist, dDAVP, was without effect. Almost all the vasopressin-sensitive neurones were localized within the facial nucleus, as assessed by histological examination of the recording sites, and about two thirds of them could be activated antidromically following stimulation of the genu of the facial nerve. We conclude that in the newborn rat, facial motoneurones are excited by exogenous vasopressin acting on V_1 receptors rather than on V_2 (renal-type) or oxytocin receptors.

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OXYTOCIN RECEPTORS IN THE GUINEA PIG OVARY DETECTED BY AUTORADIOGRAPHY.

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In mammals, oxytocin plays a key role in various functions insuring the conception, the birth and the early postnatal life of the offspring. The recent availability of a radioligand, ^{125}I-OTA, possessing high specific activity, affinity and selectivity for oxytocin receptors led us to search for oxytocin binding sites in the ovary of the guinea pig. Two-months-old guinea pigs, whose vaginal smears had been examined throughout at least two estrous cycles, were killed either at late proestrous, at estrous or at early diestrous. Binding sites for oxytocin were detected in the ovary at all stages of the cycle. The specificity of binding was ascertained by its displacement by an excess of non-radioactive oxytocin. The density of binding varied during the cycle, with a maximum at proestrous, i.e. shortly before ovulation. On emulsion-coated sections, the silver grains were associated predominantly with the theca externa, which is known to contain fibroblasts and smooth muscle cells (Burden, Am. J. Anat., 133, 125-142, 1972). The present study demonstrates the presence in the ovary of the guinea pig of binding sites, where oxytocin might act on smooth muscle cells to promote extrusion of the ovum at ovulation.

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LOCALIZATION AND CHARACTERIZATION OF VASOPRESSIN BINDING SITES IN THE HUMAN PITUITARY GLAND.

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In the present study, the distribution of vasopressin (AVP) binding sites in the human pituitary was compared to that of corticotrophs. In addition, we assessed whether human pituitary AVP binding sites resemble known AVP receptor subtypes. Pituitaries from twelve human subjects, frozen 6-24 h post-mortem, were incubated with 1.5 nM [^{3}H]AVP and processed for in vitro light microscopic autoradiography; adjacent sections were fixed in 4 % paraformaldehyde and treated to reveal ACTH immunoreactivity. Specific binding sites for AVP - displaceable by cold AVP - formed patches throughout the anterior lobe. The distribution of ACTH-immunoreactivity coincided with that of AVP binding sites. Competition studies suggested that [^{3}H]AVP binding in the human pituitary differed from that expected for both V_1- and V_2-type AVP receptors. Thus binding was not prevented in the presence of either a V_2 agonist (dDAVP), of a V_1 agonist ([Phe^2,Orn^8]VT) or of five V_1/V_2 antagonists. These results suggest that AVP binding sites in the human pituitary, thought to be involved in the control of ACTH release, may differ from V_1 and V_2 vasopressin receptors.

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DISTRIBUTION OF FIBER TYPES IN INTRINSIC FINGER MUSCLES OF THE MONKEY (Macaca fascicularis)

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In the context of our research on the control of force in the precision grip, we have investigated the histochemical specialization of intrinsic finger muscles. The distribution of fiber types was determined in 6 muscles of 4 hands (3 monkeys) on the basis of the myofibrillar ATPase activity. Main findings are: - The 6 muscles studied are heterogeneous but have in general a larger population of fast contracting type IIA and IIB muscle fibers with >80 % in abductor pollicis brevis and in first dorsal interosseus, >70 % in flexor pollicis brevis and adductor pollicis and ~60 % in lumbricalis (LUM). In the opponens pollicis (OPP) this percentage varies between 60 and 75 %. - The ratio of fiber types show significant variations between samples, except for LUM and OPP when only fiber type I and II were considered - Significant differences were also found between the four hands. These observations suggest that the majority of the intrinsic finger muscles in the monkey hand have fast contractile properties in contrast to findings in man.

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VASOPRESSIN mRNA IN THE GUINEA PIG BRAIN DETECTED BY IN SITU HYBRIDIZATION.

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Vasopressin immunoreactivity was discovered in the auditory brainstem of female guinea pigs (Dubois-Dauphin et al., Brain Res., 496 : 45-65, 1989). However, the cellular (or pericellular) location of this immunoreactivity and its local origin remained uncertain. Vasopressin mRNA was therefore searched in the female guinea pig brain by in situ hybridization (ISH) using biotinylated probes and streptavidine alkaline phosphatase cytochemistry. Reactive cell bodies were found in the hypothalamus (paraventricular, supraoptic, suprachiasmatic nuclei) and in three structures of the brainstem : inferior colliculus, periolivary zone and nucleus of the lateral lemniscus. The former two structures contain detectable amounts of vasopressin immunoreactivity but not the latter. In summary, local groups of vasopressin-synthesizing neurones - distinct from the classical hypothalamic vasopressin-producing neurones - are detected by ISH in the auditory brainstem of the guinea pig brain and may play a role in auditory information processing.

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A-CURRENTS IN TUBEROMAMMILLARY HISTAMINE NEURONES

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Histamine neurones in the posterior hypothalamus of the rat display a long-lasting transient outward rectification. We have now characterized the currents underlying this phenomenon with single electrode voltage clamp in slices. The transient outward current was inactive at resting potential, removal of inactivation was time- and voltage-dependent. The decay of the current was best fitted by two exponentials with time constants of 100 ms (IAf) and 600 ms (IAs). Only IAf was blocked by 1 mM 4-AP. Reversal potentials were -110 mV for IAf and -92 mV for IAs in 2.5 mM K+. Activation of IAs occurred 10 mV positive to that of IAf while removal of inactivation of IAs was 10 mV negative to IAf. Thus histamine neurones have two transient outward currents with different inactivation kinetics, 4-AP sensitivity, reversal potentials and voltage dependence of inactivation.

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Facilitation of the H reflex during a reaction time task and somato sensory evoked potentials to the H reflex stimuli.

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If an H reflex of the soleus muscle is elicited by posterial tibial nerve stimulation during a reaction time task, the H reflex begins to increase 60ms before the onset of a conditioned plantar flexion. This H reflex facilitation is due to reduced presynaptic inhibition of the Ia afferent endings contacting the motoneurones. Ia afferents project also to the cortex via relais stations at the origin of the dorsal spinocerebellar tract, in nucleus Z and in the thalamus. In order to test whether presynaptic inhibition is also reduced before movement onset on the way to the cortex, we recorded sensory evoked potentials (SEPs) from the scalp above the postcentral area (reverence interconnected ears and shoulder) and from the neck (reverence shoulder). The SEPs were triggered by the H reflex stimulus which was delivered at various intervals before the onset of a voluntary plantar flexion as above. Because the recordings were contaminated by the visual evoked potential (VEP) of the go signal, the VEP was computed from trials without H reflexes and then it was subtracted from each test recording. The recordings were grouped according to their occurance before movement onset and then averaged. The first data give some support to the hypothesis that early SEP components increase in parallel with the premovement H reflex facilitation.

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NEUROTOXIC EFFECTS OF BISMUTH IN VITRO

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In the present study effects of bismuth (Bi) on serum free fetal chick neuronal retina, total brain, brainstem and meninges monolayer cell cultures and on cultured rat hippocampal slices were investigated. No acute effects of Bi (1-100 uM Bi) on the hippocampal slice cultures were detected, using electrophysiological techniques. However, after long term application of Bi, signs of a change in viability and differentiation were already seen between 1 and 10 uM in all cultures tested. At those Bi concentrations present in blood Bi encephalopathy occurs in humans and mice. From human data it is known, that Bi is accumulated in the brain, especially in the brainstem. In line with these data monolayer cultures of the chick brainstem were more sensitive to Bi in comparison to cultures of the total brain. In total brain and in brainstem cultures astroglia, in comparison to nerve cells were affected at significant lower Bi concentrations. The distribution of Bi in the cells was checked by electron probe X-ray analysis.

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3H-NECA binding: A comparison between Spontaneously hypertensive (SHR) and normotensive (WKY) rats.

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Stress has been shown to affect adenosine binding (1) and Maudsley-reactive (MR) and Maudsley-non-reactive (MNR) rats differ in adenosine binding (2). Since SHR and WKY rats differ in behavioral and hormonal responses upon being exposed to a novel environment, too (3), we compared 3H-NECA binding between these 2 genetically distinct rat-strains. At 4nM, SHR exhibited a significantly higher binding than WKY rats in both whole brain homogenate (+21%) and in diverse CNS-subregions (+10 to +26%). Scatchard-analyses revealed interstrain differences for Bmax- but not Kd-values. Additional studies (in the presence of 50nM CPA or with 3H-CHA as ligand) pointed to dissimilar numbers of A1-sites. Our present data (and those previously reported for MR/MNR rats) will be discussed in relation to strain-differences in stress-susceptibility and/or blood-pressure.

(1) Anderson et al. Pharmacol.Biochem.Behav. 26:829(1987)
(2) Marangos et al. Brain Res. 421:69(1987)
(3) Gentsch et al. Physiol.Behav. 27:183(1981)

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CHARACTERIZATION OF IMMUNOGENIC OLIGOSACCHARIDES OF THE HUMAN MYELIN-ASSOCIATED GLYCOPROTEIN (MAG) AND P_0.

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Human MAG and P_0 share a common carbohydrate sequence containing a sulfated-GlcUA that is recognized by both the mouse monoclonal IgM antibody (M-IgM) HNK-1 and human M-IgM that are found in a large proportion of patients with demyelinating polyneuropathy. The epitopes for these M-IgM are borne by complex and hybrid structures of *N*-linked oligosaccharides (Burger et al. *J. Neurochem.* in press). MAG and P_0 glycopeptides were obtained by proteolysis with V_8 protease or trypsin respectively. The analysis of MAG and P_0 glycopeptides by serial affinity chromatography on immobilized lectins (ConA, E_4-PHA, PSA, L_4-PHA, DSA, and RCA1) showed that both glycoproteins display a high degree of heterogeneity in their oligosaccharide structures but the epitopes for HNK-1 and patient's M-IgM were found mainly on *N*-linked oligosaccharides that contain a fucose residue in the core.

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REGULATION OF THE DUPLICATED MYELIN BASIC PROTEIN GENE EXPRESSION IN MYELIN DEFICIENT MICE

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Myelin basic protein (MBP) gene is duplicated in myelin deficient (mld) mice. The two genes are in tandem position, and the upstream gene is partially inverted. Our previous experiments showed extremely low concentrations of MBP and MBP-specific mRNA in mld brains. The transcription rate of each Mbp gene was measured directly in isolated nuclei using strand-specific RNA probes corresponding to part of the inverted sequence. Both genes are transcribed from their own promoters. Dot blot analysis of nuclear and cytoplasmic RNAs showed the presence of antisense MBP-specific RNA in mld brain nuclei. Reduced amounts of MBP RNAs are present in both nuclei and cytoplasm. Nuclear MBP mRNA concentration is about 5 times higher than that of cytoplasmic MBP mRNA. Our results suggest that the Mbp gene is inhibited post-transcriptionally by its own antisense RNA, which interferes with normal RNA processing or/and transport to cytoplasm. Northern blot analysis showed maximal mld Mbp gene expression at 40 days of age, in contrast to about 20 days of age in wild type mice. Nuclear MBP RNA concentration showed the same peak for both sense and antisence RNAs. Therefore, both Mbp genes are abnormally and similarly regulated in mld mutant mice.

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INTRACEREBRAL SOURCE LOCALIZATION OF FFT-TRANSFORMED MULTICHANNEL EEG DATA

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Intracerebral localizations of EEG sources are of interest in brain research and clinical diagnostics; momentary spatial distributions of the brain electric field (multichannel maps) are used. However, normal and pathological brain states often are assessed using Fourier-transformed EEG (FFT); but, power maps are not suitable for dipole source localizations, since polarity information is lost. We describe a method to generate (from the sine-cosine coefficients of the FFT) a voltage distribution map which can be used for the dipole source location: A best-fit straight-line is fitted into the FFT's sine-cosine diagram for each frequency component; all electrode points are projected onto that line; the amplitude and polarity of the projected points vs the average reference is determined. For averaging of results over time epochs or subjects, the polarities have to be permutated for the least sum of standard deviations for all electrodes. Dipole source locations for each frequency point can then be computed. Data of patients with brain damage and of normal subjects during different brain states illustrate the validity of this strategy.

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TOPOGRAPHIC RECOGNITION OF ERP COMPONENTS AFTER SCOPOLAMINE

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Event-related potential (ERP) components index human information processing. Drug-induced latency changes can clarify which processes are affected. ERP maps were recor-ded in a double-blind study of 12 subjects before and after scopolamine and placebo. Topographic component recognition identified components as invariant ERP landscapes to improve latency estimates. Estimated model maps were used to search each ERP for the most similar map. Iterative search resulted in stable solutions with high average correlations between model and individual maps (P3: r=.89-.93). P3 map latencies (ca. 400 ms) changed with task conditions like conventional P3 latencies, but revealed additional delays after scopolamine, without landscape changes. Topographic latency measures are thus both interpretable and sensitive.

Scopolamine-induced delays of the visual N1 (160-210 ms) preceded P3 slowing. This was especially marked for peripheral high spatial frequency gratings. "Hidden" peripheral targets were least affected. Scopolamine seems to interfere with an attention response to peripheral stimuli.

(1) Now: Neurology, Universitaetsspital, 8091 Zurich.

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DIURNAL TYPOLOGY: IMPLICATIONS FOR CHRONOBIOLOGICAL RESEARCH

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In recent chronobiological literature the morningness-eveningness dimension has been connected with sleep-wake rhythm, body temperature, alertness and varied adaptability to shift work and jet lag.
The present investigation, including 20 healthy subjects (11m,9f;age range:23-52 years), studied the interaction between the 'diurnal type' (Morningness-Eveningness Scale from Horne and Oestberg,1976) and a set of variables governed by circadian oscillators and/or implicated in chronobiological mood and sleep disorders.
Results indicate, that a low score in the Horne-Oestberg Index (Eveningness) is related to small nocturnal urinary volume, high nocturnal urinary Melatonin, high daytime fatigue, sadness and sleep disturbance and a high proportion of females. Associated there-with a high degree of self-rated seasonality (Seasonal Pattern Assessment Questionnaire, Rosenthal et al., 1984).
These 'diurnal type'related features may contribute to inter-individual differences in adjustment to environmental changes and to a differential vulnerability to sleep and mood disorders affected by season.

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EFFECT OF SLEEP STATE ON CEREBRAL HAEMOGLOBIN (Hb) VOLUME IN TERM NEWBORN INFANTS ASSESSED BY NEAR INFRARED SPECTROSCOPY (NIRS)

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NIRS can be used for monitoring cerebral haemodynamics and oxygenation in newborn infants. The AIM of this study was to compare cerebral Hb volume (Hb-tot = Hb-ox + Hb-red) between quiet and active sleep. METHOD: NIRS was performed on 18 healthy term infants (birthweight: 2570 to 4150g, age: 2 to 8 days). TcpCO2, tcpO2, SO2 and heart rate were recorded and sleep states assessed according to Prechtl (Brain Res 76,p185,1974). RESULTS: (values are median (range) umol/l): After transition from quiet to active sleep Hb-ox decreased: -3.5 (0 -10) p<0.001 and Hb-tot:-4 (+2 -10) p<0.001. From active to quiet sleep Hb-ox increased: +3 (0+16) p<0.001 and Hb-tot +4 (0 +18) p<0.001. Heart rate increased by 9 bpm (p<0.001) from quiet to active sleep while Hb-red, tcpO2, tcCO2 and SO2 did not change significantly.
CONCLUSION: These findings demonstrate that sleep state changes correlate with changes in cerebral Hb volume (around 5%) and therefore must be taken into account during studies of cerebral haemodynamics.

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MODULATION OF EXTRACELLULAR ASPARTATE LEVEL DURING EPILEPTIFORM EVENTS IN PRIMARY FOCI OF PATIENTS

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Many evidences support the role of excitatory amino acids (aa) in animal models of epilepsy. In the course of presurgical Stereo-EEG evaluation of candidates for epilepsy surgery, extracellular fluid from the primary foci was analyzed by HPLC for putative aa transmitters. A push-pull cannula was introduced into the lumen of a standard hollow core multi-contact depth probe placed in the hippocampus. A computer-assisted analysis of the background depht-EEG and of spontaneous and electrically provoked epileptiform events were performed and the results were compared with the biochemical measurements. In two out of three operations, an increase of aspartate level was observed, correlating with epileptiform EEG events. Non-transmitter aa remained unaffected.

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EVIDENCE FOR FLUOROGOLD UPTAKE AND SUBSEQUENT RETROGRADE TRANSPORT IN UNDAMAGED FIBRES OF PASSAGE IN THE RAT

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Fluorogold (FG) has been introduced by Schmued and Fallon (1986) as a new retrograde tracer with special properties, one of which is that no tracer uptake by undamaged fibres of passage occurs. Since this special property is critical for the suitability of FG tracing particularly in double labeling studies, the dorsal columns with their strict somatotopic organisation were chosen as a model in which a potential uptake by undamaged fibres of passage could be assessed. Injections into the gracile fasciculus (GF) centered near the posterior median septum resulted in FG labeling not only in somatotopically related ipsi- and contralateral lumbar DRG cells, but also in thoracic and even cervical DRG cells bilaterally. Injections centered either between the GF and cuneate fasciculus (CF) or into the CF, revealed FG activity in all ipsi- and contralateral lumbar DRG cells. Since the pipette diameter was always considerably thiner than the width of GF or CF and the initial tracer diffusion area, seen 2 hours after the injection, covered both dorsal columns it is presumed that not only mechanically damaged fibres but also undamaged ones took up and retrogradely transported FG.

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A PROCEDURE TO PROJECT TOPOGRAPHIC MAPS IN THE BRAIN FROM CURVED SURFACES INTO A PLANE

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Topographic maps form a dominant feature in the brain. They are often projected onto surfaces which are curved in many different ways. One would like to be able to compare the topography on these surfaces quantitatively with the origin of the map. This comparison would be possible if the curved brain surfaces could be represented in planes. Therefore, we had to develop a procedure for unfolding maps on convex structures. For this purpose a unit sphere was constructed whose origin was close to the centre of gravity of the curved brain structure. Then the surface of this structure was projected into the sphere. From its representation on the spherical surface it was unfolded into the plane using a polar azimuthal radially equidistant projection. Our method can safely be applied to any topographic map as long as it is represented on convex brain structures.

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LOCALIZATION OF PROSTAGLANDIN E_2 HIGH AFFINITY BINDING SITES ON CHICKEN SPINAL CORD MOTONEURONES

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Chicken spinal cord possesses two types of prostaglandin E_2 (PGE_2) binding sites. A low affinity (K_d : 2 µM), high capacity PGE_2 binding site and a high affinity (K_d: 1nM), low capacity PGE_2 binding site. Cellular distribution of PGE_2 binding sites in cryostat section of spinal cord was investigated with light microscopic radioautography, after incubation with 3nM ^3H-PGE_2. Beside scattered silver grains, a high concentration of bound radioactivity was associated with mononeurones. Since this strong radioautographic reaction was completely inhibited by addition of 10 nM unlabeled PGE_2, it is postulated that motoneurones possess high affinity PGE_2 binding sites.

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COUPLING OF PROSTAGLANDIN E_2 LOW AFFINITY BINDING SITES TO ADENYLATE CYCLASE STIMULATION IN THE CHICKEN SPINAL CORD.

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Prostaglandin E_2 (PGE_2) is formed in spinal cord. PGE_2 interacts with two classes of binding sites (K_{d1} = 1 nM, K_{d2} = 2 µM). To specify the transduction mechanism, the role fo PGE_2 on the adenylate cyclase system was investigated. After pretreatment with pertussin toxin, 0.1-50µM PGE_2 enhanced the level of cAMP in spinal cord fragments and potentiated the forskolin response in a dose dependent manner. The apparent activation constant (1 µM) was closely related to K_{d2} of PGE_2 binding sites. These results indicate that the low affinity PGE_2 binding sites are coupled to the stimulation of adenylate cyclase.
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VOLTAGE DEPENDENCE OF Na CHANNEL INACTIVATION IN THE SQUID GIANT AXON

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We have reported a slow gating current I_g in low-noise recordings from the squid giant axon (Forster and Greeff, J.Physiol.,July '89 meeting) and developed an analytical technique allowing us to check if it is due to open to inactivated (O-I) transitions alone and thus seen as $I_{g,h}$ resulting from a voltage dependent inactivation gate with quantal charge q_h. The novel isochronic analysis of I_g vs $\dot{g}_{Na}$ enabled us to identify $I_{g,h}$ and together with the single channel conductance to obtain the quantal inactivation gating charge $q_h = 1.25\ e^-$ per O-I transition. The apparent inactivation time constant τ_h appeared related to the O-I transitions only at voltages (V_p) > -10 mV but below it is also determined by the closed to open transitions. Therefore the conventional rate analysis of $\ln(\tau_h)$ vs V_p will give different values for q_h in different V_p regions. When applying rate analysis with these constraints we find $q_h = 1.19\ e^-$ which corroborates the 1.25 e^- obtained by the totally different isochronic method. In conclusion i) a rather accurate figure for the quantal inactivation gating charge is obtained which ii) can be used to test molecular dynamical models and iii) the divergent figures for q_h from previous rate analyses appear to result from uncertainties about the constraints reported here (Supported by Swiss NF grant 3.143-0.85).

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CHARACTERISTICS OF THE FAST DISPLACEMENT CHARGE IN THE SQUID GIANT AXON.

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The fast displacement charge movement previously reported in squid giant axons (Biophys. J., 55,316a) has been studied with improved time resolution (0.5 µs/point) and precautions taken to ensure smooth settling of the true membrane potential. Using a voltage step protocol having a variable-width depolarizing step to 20 mV, we have recorded the displacement charge which flows following an off-step to -100 mV. Analysis of these data show that the amount of fast charge returned (1-2 nC/cm^2) and its relaxation time constant (10-20 µs) are independent of the depolarizing pulse width and are in addition unaffected by the presence of an inactivating pre-pulse to 0 mV for 20 ms. The Q-V and τ-V curves for this component exhibit the characteristics normally associated with a two state process. These results allow us to exclude distortion of the main gating charge movement resulting from inhomogeneous membrane charging as a source for this fast component. Furthermore, instrumentation asymmetries account for < 20% of the charge involved. This substantiates our conclusions that it is a displacement charge acting independently and in parallel with the main sodium channel gating charge. (Supported by Swiss NF grant 3.143-.0.85.)

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MULTISITE OPTICAL RECORDING OF ELECTRICAL ACTIVITY IN CEREBELLAR SLICE CULTURES

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To understand information processing in neuronal networks it is necessary to record bioelectrical activity simultaneously from multiple sites within a network of nerve cells.
We applied optical methods using voltage sensitive dyes to organotypic cultures of rat cerebellum and co-cultures of cerebellum and inferior olive. These cultures offer unique opportunities since optical signals with a high signal-to-noise ratio can be recorded from single neurons integrated in organotypic synaptic networks.
We recorded optical signals with an array of 10x10 silicon photodiodes which was placed in the image plane of a 40x objective and mounted on an inverted microscope (Zeiss Axiovert).
Cultured Purkinje cells spontaneously displayed synaptic and spiking activity which could be recorded at the single cell level from several neurons simultaneously. Monopolar stimulation within cerebellar cultures elicited graded postsynaptic potentials. In contrast, olivary stimulation evoked characteristic climbing fiber-like responses in Purkinje cells.

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REORGANIZATION OF CORTICOSPINAL PROJECTIONS AFTER NEONATAL CORTICAL LESIONS IN THE RAT.

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Corticospinal neurons of the rat sensori-motor cortex project almost exclusively to the contralateral spinal cord. Total or semi-hemispherectomy at birth induces an increase of ipsilateral corticospinal projections originating from the opposite intact cortex. The aim of this study was to establish whether a similar reorganization of corticospinal projections occurs as a result of a restricted neonatal lesion of the motor cortex, and if so, to evaluate quantitatively the increase of ipsilateral projections from the opposite intact cortex in comparison with normal animals. The neuroanatomical tracer WGA-HRP was injected in lesioned rats either in the spinal cord or in the motor cortex (forelimb area), both on the opposite side of the lesioned cortex. Similar injections were performed in normal rats. Counts of retrogradely labeled neurons in the ipsilateral motor cortex with respect to the spinal cord injection and densitometry measurements of anterogradely labeled terminals in the spinal cord after cortical injection showed a 10 fold increase of the proportion of ipsilateral corticospinal projections in the lesioned rats in comparison with control animals. The present work supports quantitatively the existence of an important plasticity of the corticospinal projections, even as a result of relatively small neonatal cortical lesions.

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CHANGES IN ELECTRICAL PROPERTIES OF RABBIT VAGUS NERVE DURING WALLERIAN DEGENERATION AND REGENERATION.

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Adult rabbits were anaesthetized and their left vagus nerve transected at a high cervical level at two points located 6 to 8 cm apart. At different times after the operation the rabbits were killed and the operated nerve stump (ON) and the intact nerve (IN) removed. The electrophysiological properties of both ON and IN were studied by the sucrose-gap technique. The action potential (AP) in ON decreased by 50% and 75% after 3 (n=3) and 6 (n=2) days, respectively. After 10 days (n=5) APs in ON were absent. After 15 days or more (n=17), the membrane potential (E_m) of the remaining elements of the ON (presumably Schwann cells) still depended on external K^+, as it does in INs. On the other hand, increasing external Ca^{2+} depolarized IN while it hyperpolarized ON. Moreover, ACh, 5-HT and veratrine, which depolarized IN, were without effect on ONs. Unexpectedly, a brief (0.4ms) depolarizing pulse, which elicited an AP in the INs, produced a slow depolarizing wave in the ON, whose mean amplitude was about 1mV, and lasted 3-5min. In ON removed from rabbits 43 days after the operation (n=3), slight APs (0.2% of APs in IN) were observed, suggesting the presence of regenerating axons. This was indeed confirmed by a light- and electron-microscopic analysis of nerves removed 57 days after the operation, and in which the APs were 3% of the APs in IN (n=4).

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RAT CORTICOSPINAL TRACT (CST) FIBERS REGENERATE IN THE ABSENCE OF NEURITE GROWTH INHIBITORS

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Adult CNS tissue has been shown to contain potent inhibitors of neurite growth (NI-35, NI-250) localized in CNS myelin (Schwab and Caroni, J. Neurosci. 8: 2381). To study their role in neurite regeneration, myelination was completely suppressed by X-irradiation of newborn rats, or, alternatively, neutralizing antibodies to NI-35 and NI-250 (Caroni and Schwab, Neuron 1: 85) were applied through the CSF. The dorsal 2/3 of the spinal cord (including the CST) were transsected in these rats at 2-6 weeks of age. After 2 - 3 weeks the corticospinal tract was traced by anterograde labeling (WGA-HRP) and reconstructed from serial sagittal sections. In control animals sprouting caudal to the lesion occurred over 0.2 - 1 mm from the lesion site. In contrast, myelin-free and antibody-treated animals both showed elongation of regenerating CST axons over 5 - 7, in a few animals over 10 mm caudal to the lesion. The number of regenerating fibers was small, probably due to cysts and scars at the lesion site. These results demonstrate a crucial role of neurite growth inhibitors for the lack of axonal regeneration in the adult CNS.

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THE CLONED RAT β1-SUBUNIT OF THE $GABA_A$-RECEPTOR IS SUFFICIENT TO FORM A RECOGNITION SITE FOR PICROTOXIN

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We presently investigate the structural basis of GABA gated chloride channels in mammalian brain, using functional expression of cDNAs coding for the different subunit isoforms. In this context, we expressed individual cloned cDNAs coding for subunit isoforms of the rat $GABA_A$ receptor in the Xenopus oocyte. Only in the case of the β1-subunit functional expression of an ion channel was found. This channel was anion-selective and opened in the absence of GABA. This non-gated anion conductance could be closed by the GABA channel blocker picrotoxin in a dose-dependent manner (K_i = 1 μM). Co-expression of an α-subunit (α1, α3 or α4), with the β1-subunit prevented formation of non-gated channels. Instead, GABA gated ion channels were formed. We conclude that the β1-subunit of the $GABA_A$ receptor is sufficient to form a binding site for picrotoxin.

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NEURAL INDUCTION OF ACETYLCHOLINE RECEPTOR SPECIFIC mRNA AT RAT NEUROMUSCULAR SYNAPSES.

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In mammalian muscle the subunit composition of the nicotinic acetylcholine receptors (AChR.s) is developmentally regulated and the AChR.s become concentrated in the synaptic muscle membrane as the synapse matures. We have used in-situ hybridization techniques to examine the synaptic localization of the mRNA.s encoding the α- and ε-subunits of rat muscle AChR. The localization of α-subunit mRNA at the synapse seems to arise from its selective disappearance from the nonjunctional fibre segments due to the onset of muscle activity induced by innervation. In contrast, the ε-subunit mRNA appears only postnatally and locally at the site of the synapse; denervation at the day of birth does not prevent its later expression at the synaptic site. The accumulation of autoradiographic grains around endplate nuclei suggests that synaptic ε-mRNA accumulation is induced by a locally restricted signal from the nerve acting selectively on the junctional subpopulation of muscle nuclei very early in synapse formation. These data suggest completely different regulatory mechanisms for the expression of various AChR subunits.

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THE AFTER-POTENTIALS IN MAMMALIAN NERVE FIBRES: ARE SCHWANN CELLS INVOLVED?

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The action potential (AP) and the following after-potentials of rabbit vagus nerve were measured by the sucrose-gap method. Three kinetically distinguishable after-potentials were observed: a fast and a slow hyperpolarizing after-potential (fHAP and sHAP) and a depolarizing after-potential (DAP). The fHAP were greatly diminished by low concentrations of 4-AP (10 μM) or dendrotoxine (100nM). This decrease in fHAP was accompagned by an enhancement of the sHAP. Both the fHAP and the sHAP were enhanced by an increase in the K^+ electrochemical gradient. The sHAP was, furthermore, composed by a Ca-sensitive part and a Ca-insensitive one. The Ca-sensitive component was blocked by TEA 1mM. When K_{out} was increased, the HAP was replaced by a DAP, insensitive to TEA, but entirely dependent on the presence of Ca_{out}. The above observations suggest that the fHAP corresponds to the tail of the delayed rectifier K^+ current, while the Ca-sensitive part of the sHAP is probably mediated by a Ca-activated K permeability. The origin of the two other after-potentials, the Ca-independent component of the sHAP and the DAP, are more difficult to explain. The fact that they persisted even when the axonal conduction was blocked by TTX, indicates that they could reflect an electrical activity of the Schwann cells, which are the most numerous satelite cells in this preparation. Results obtained on Schwann cells from degenerated nerves argues in favour of this hypothesis (Brunet *et al.* in this issue).

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Release of acetylcholine from Xenopus oocytes injected with mRNA from cholinergic neurons.

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Poly(A)+mRNA extracted from the electric lobe of the Torpedo fish was injected into Xenopus oocytes. Cholinergic proteins such as choline acetyl-tranferase were expressed with a maximum at the 4th day. The injcted oocytes synthetized Ach from radiolabelled and unlabelled precursor. We stimulated such oocytes by A23187 or KCl and demonstated calcium-dependent transmitter release. By immunoblotting it was shown that the electric lobe mRNA injcted oocytes expressed the mediatophore, a membrane protein able to translocate Ach.

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A NEW METHOD TO MEASURE CYTOSOLIC FREE CALCIUM USING FURA-2 IN NERVE CELLS

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In order to study cytosolic free calcium, $[Ca^{2+}]_i$, in nerve cells, we used the highly fluorescent calcium indicator fura-2.
Upon binding calcium, the excitation spectrum of fura-2 exhibits a considerable shift towards shorter wavelengths. This effect allowed us to calculate $[Ca^{2+}]_i$ by the ratio method described in the literature. This approach reduces the signal-to-noise ratio, since excitation wavelengths must be switched periodically, resulting in a reduction of the exposure time per measurement at a single wavelength.
Therefore we have developed a new strategy to quantitate time courses of $[Ca^{2+}]_i$ recorded at one excitation wavelength only. We made use of a linear relationship that can be established between the two fluorescence intensities at constant $[Ca^{2+}]_i$, optical path length and illumination intensity. Thus, we were able to calculate the fluorescence ratio and hence $[Ca^{2+}]_i$ from a time course experiment monitored at one wavelength only with both high spatial and temporal resolution.

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ACTION OF GLUTAMATE ON THE NEUROPILE ARBORIZATIONS OF LOCUST MOTONEURONS.

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In locusts, there is evidence that glutamate is a centrally released neurotransmitter. To establish the pharmacological profile of putative synaptic glutamate receptors on central neurons, the effect of pressure applications of various glutamate agonists was recorded from neuropile impalement sites in flight motoneurons. The prevalent response caused by glutamate was an inhibition of spontaneous activity associated with an increase in a chloride conductance. In different cells, the response was hyperpolarizing or depolarizing at resting potential but always hyperpolarizing at spiking threshold. In a small number of cells, glutamate triggered a biphasic response consisting of a depolarization superimposed on a hyperpolarization. NMDA and glycine were without effect while kainate and quisqualate caused large depolarizations and spiking, even in cells inhibited by glutamate. Ibotenate triggered large hyperpolarizations accompanied by a conductance increase, with a reversal potential similar to that of the glutamate inhibitory response. Aspartate mimicked all the effects of glutamate. These data suggest the presence of an inhibitory glutamate receptor for which aspartate and ibotenate are agonists and, possibly, a non-NMDA excitatory receptor.

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CGP 35 348: A NEW $GABA_B$ RECEPTOR BLOCKER CROSSING THE BLOOD-BRAIN BARRIER

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CGP 35 348 (P-(3-aminopropyl)-P-diethoxymethyl-phosphinic acid) has an IC_{50} of 39 µM in a $GABA_B$ receptor assay. In many other assays there is no interaction at 1mM i.e. the compound is very selective.
CGP 35 348 (10, 100 µM) antagonizes the depressant effects of baclofen (0.5-10 µM) on neurons in a variety of in vitro models including, the spinal cord, the hippocampus and locus coeruleus. CGP 35 348 (10, 30 µM) blocks the late inhibitory postsynaptic potential in CA1 pyramidal neurons. CGP 35 348 appears to be 10 to 30 times more potent than the $GABA_B$ receptor blocker phaclofen. CGP 35 348 enters the brain after intravenous and intraperitoneal but not after oral administration. In the awake rats CGP 35 348 (30, 100 mg/kg i.p.) antagonizes the motor-impairing effect of baclofen in the rotarod test. This drug should be of considerable value in elucidating the role of brain $GABA_B$ receptors.

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TRYPSINE TREATMENT OF *TORPEDO* SYNAPTOSOMES INHIBITS ACETYLCHOLINE RELEASE AND DEGRADES A MEMBRANE ANTIGEN POSSIBLY INVOLVED IN RELEASE.

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A monoclonal antibody that is able to inhibit acetylcholine (ACh) release in *Torpedo* synaptosomes recognizes an antigen which exists as a monomer (65 kDa) and a dimer (135 kDa) in the synaptosomal plasma membrane. By immunoblotting analysis we observed that treatment of synaptosomes with increasing concentrations of trypsine (25 to 200 µg/ml) induced a progressive degradation of these two polypeptides into lower molecular weight components. Also, a progressive decrease of ACh release (from 5 to 50%) was produced. However, complete inhibition was never reached. Treatment of synaptosomes with proteinase K inhibited 20 to 30% of the release but did not significantly degrade the antigen. The 135 kDa and/or the 65 kDa polypeptides might be directly involved in the ACh release mechanism.

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HYDROPHILIC AND AMPHIPHILIC CHOLINE ACETYLTRANSFERASE IN DROSOPHILA MELANOGASTER

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The specific activity of choline acetyltransferase (ChAT) increases during the development of the Drosophila. Using the Triton X-114 partition method, amphiphilic ChAT activity was measured in Drosophila; it reached 4% of total ChAT activity in embryo and 12% in larvae, pupae and adult flies. The sedimentation coefficients of amphiphilic and hydrophilic ChAT from crude preparations of the enzyme were 4.8S and 5.2S corresponding to molecular weights of about 69 and 77 kDa, respectively (catalase being used as standard). Removal of the detergent from the amphiphilic ChAT containing samples led to a large decrease of the enzyme activity. Aiming to clone a cDNA coding for amphiphilic ChAT, oligonucleotides directed towards two regions of the ChAT protein were synthetized on the basis of the cDNA sequence known for hydrophilic ChAT. These oligonucleotides were used to screen a λgt10 Drosophila genomic library. A EcoR1 fragment of 5.2 kb was cloned which hybridized "in situ" with the 91BD region of the polythene chromosome 3R from the Drosophila salivary gland; this region corresponds to the locus for the gene of ChAT. This fragment is now being sequenced and used for screening cDNA libraries.

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DEVELOPMENT AND APPLICATIONS OF 4 SPECIFIC ANTI-NPY MONOCLONAL ANTIBODIES.

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Neuropeptide Y (NPY) is a 36 aminoacid peptide found throughout the central and peripheral nervous system where it is costored with norepinephrine. NPY presents structural homologies with a family of peptides (PYY, PP). The radioimmunological determination of plasma NPY is complicated by cross-reactions with these peptides. To improve the immunological detection of NPY in biological samples, 4 anti-NPY monoclonal antibodies (Ab) have been produced and their epitopes precisely characterized. These Abs were used to detect specifically NPY in neuroendocrine tumors by immunohistochemistry and to develop a sensitive two-sites immunoradiometric assay for mature NPY. With this assay, no cross-reactivity with PYY or PP is found and the detection limit is at 0.5 pmol/L. Plasma NPY levels might be a marker of interest in some neuroendocrine tumors. Indeed, elevated NPY levels were found in patients with malignant or begnin phaeochromocytoma, a tumor derived from adrenal chromaffin cells.

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PRESENCE OF THE Ca^{2+} BINDING PROTEIN PARVALBUMIN IN THE GERBIL HIPPOCAMPAL ENTORHINAL PROJECTION.

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Parvalbumin (PV) is a cytosolic protein with high affinity Ca^{2+} buffering activity. In the CNS it is considered as the biochemical correlate of outstanding physiological properties of the subpopulation of GABAergic neurons expressing it. In the gerbil (Meriones unguiculatus), an animal used as model for ischemia and epilepsy, we found PV immunoreactivity (PV-IR) in areas corresponding to the terminal field of the excitatory perforant path. Ultrastructural analysis confirmed PV-IR in boutons with features of excitatory synapses. Unilateral ablation of the entorhinal cortex resulted in a bilateral decrease of PV staining in the molecular layer of the dentate area. Double labelling of the entorhinal cortex with PV and GABA showed their coexistence in cells with features of interneurons whereas multipolar neurons in layer II were only PV-IR.Thus it is likely that in the gerbil hippocampus PV is associated with an excitatory projection pathway.

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DOPAMINERGIC MODULATION OF NEUROPEPTIDE Y-IMMUNOREACTIVITY IN RAT STRIATUM.

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Lesion of substantia nigra (SN) pars compacta by the specific neurotoxin 6-hydroxydopamine (6-OHDA) results in a persistent increase in the number of neuropeptide Y (NPY) immunoreactive neurons in the striatum ipsilateral to the lesion (Kerkerian et al. Neurosci Lett. 66:106, 1986). We were interested to investigate whether substitution with dopamine will reverse this effect. For this purpose rats were divided in 2 groups, one of which was injected in front of SN with 6-OHDA, the other with equal amount of physiological saline. After development of dopaminergic supersensitivity in the 6-OHDA-injected animals (as tested under apomorphine in the rotometer) half of each group received a continuous infusion of methyl-L-dopa (50 or 75 mg/kg/d) via Alzed-minipump and 2 daily injections of benserazide (5 mg/kg). Two to four weeks later, rats were perfused and NPY-immunoreactivity studied in frozen sections. Extent of the lesions was verified in serial cresyl stained or anti-tyrosine hydroxylase immunoreacted sections. The results show that NPY-immunoreactivity in striatum is closely related to the degree of functional dopaminergic deafferentation. Supported by NF 31-25292.88.

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Expression of functional $GABA_A$ receptor subtypes $\alpha1\beta1\gamma2$ and $\alpha3\beta1\gamma2$ in mammalian cells.

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GABA, the major inhibitory neurotransmitter in the vertebrate brain, mediates neuronal inhibition by opening a chloride channel integral to the $GABA_A$/benzodiazepine receptor (BZR) complex. The receptor is assembled from α, β and γ-subunits which form a heterooligomer of unknown stoichiometry. To investigate the pharmacological differences in BZR subtypes we cloned the full length cDNAs coding for the subunits $\alpha1$, $\alpha3$, $\beta1$ and $\gamma2$ into the expression vector pBC12. When 293 cells were co-transfected with various subunit combinations high mRNA levels were detected and the appearance of receptor molecules on the cell surface was demonstrated by immunofluorescence using a polyclonal antibody to the native rat $GABA_A$ receptor.In electrophysiological measurements functional $GABA_A$ receptors were detected which were both GABA-sensitive and potentiated by pentobarbital when the sub-units $\alpha1\beta1$ or $\alpha3\beta1$ were co-expressed. However, potentiation of the GABA response by benzodiazepines was only observed when the $\gamma2$ subunit was included in the above combinations. A characteristic decrease of the GABA signal was observed when cells were stimulated with GABA in the presence of βCCM.

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Pharmacological modulation of the NMDA receptor by agonists and antagonists at the strychnine-insensitive glycine site

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The NMDA receptor is a multiple domain transmitter-gated neuronal membrane channel. The most potent antagonists currently defined for this receptor are CGP 37'849 (competitive), MK-801 (channel blocker), 7-chlorokynurenic acid (7CKA) and HA-966 (allosterical modulators), with K_i values of about 35 nM, 5 nM, 0.2 µM and 3 µM as determined using receptor binding techniques. We have compared the actions of these substances on NMDA-evoked responses in the rat neocortical "wedge" and hemisected spinal cord preparations. All substances dose-dependently antagonized the depolarizing action of NMDA, but with markedly different potencies (< 1µM for CGP 37'849 and MK-801; > 50 µM for 7CKA and HA-966). However, unlike CGP 37'849 and MK-801, the antagonist effects of 7CKA and HA-966 could be overcome by increasing concentrations of glycine or D-serine (a glycine-like agonist) in the perfusion medium, thus confirming their distinctive actions. Interestingly, in the spinal cord but not in the cortex, D-serine alone potentiated NMDA-evoked depolarizations; this may indicate that the NMDA receptor complex may, via differences in the composition of the extracellular environment (e.g. glycine levels), be subject to differing regulatory influences between brain regions.

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TRANSIENT POTENTIATION OF THALAMO-CAUDATE EPSP BY 5HZ STIMULATION OF CENTRO-MEDIAL/PARAFASCICULAR REGION IN HALOTHANE ANAESTHETIZED CATS, IN SITU.

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Intracellular recordings were made from neurons in the head of the caudate nucleus in halothane anaesthetized cats. Low frequency 0.5Hz stimulation of the thalamic centro-medial/parafascicular region led to an initial fast EPSP, thought to be monosynaptic due to lack of changes in latency with varying suprathreshold stimulation intenities. The initial EPSP was followed by an IPSP and 3-4 membrane potential oscillations. 5Hz stimulation strongly potentiated the initial EPSP, measured as increase of the area under the curve of the EPSP plot. Potentiation started with the 2.- 3. stimulation pulse within the high frequency train, and usually decayed after the 10.-18. pulse, without apparent resting potential changes. The 0.5Hz level was usually reached within the first 25 5Hz pulses. In some cells, the EPSP remained potentiated over more than 50 pulses. Potentiation could be due to superposition of the initial fast EPSP with a following oscillation. These properties of the thalamo-striatal pathway could provide a frequency filter.

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N-ACETYL-ASPARTYL-GLUTAMATE RELEASE IN VITRO FROM RAT STRIATUM, SPINAL CORD AND CEREBELLUM

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N-acetyl-aspartyl-glutamate (NAAG) has been proposed as a transmitter in the mammalian CNS. Depolarization induced release of NAAG has been investigated in slices of rat striatum, cerebellum and spinal cord. The superfusates were analyzed by gas chromatography mass spectrometry with chemical ionization and selected ion monitoring. The 50 mM K^+-induced release (and the % Ca-dependency) amounted to 10.3 ±3.0 pmol/mg protein.10 min in the striatum (89%), 22.0± 5.2 in the cerebellum (99%) and 19.2±7.6 in the spinal cord (65%). In the cerebellum, the degeneration of the climbing fibers induced by 3-acetyl-pyridine resulted in a 49% decrease in the NAAG release.

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MAGNESIUM REDUCES THE CONDUCTANCE OF NEURONAL NICOTINIC ACETYLCHOLINE RECEPTORS OF RAT PHEOCHROMOCYTOMA (PC12) CELLS.

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Following short term treatment with nerve growth factor, the rat tumor pheochromocytoma cell line PC12 expresses neuronal nicotinic acetylcholine receptors (NnAChRs) consisting of α_3 and β_2 subunits. The properties of individual NnAChRs were examined in excised outside-out membrane patches. Both sides of the membrane faced a solution consisting of (mM): CsCl, 150; $MgCl_2$, 1.2; HEPES, 10; EGTA, 5. The effect of Mg^{2+} on single channel conductance was investigated by modifying its concentration on either side of the patch. Increasing the internal Mg^{2+} concentration reduced the outward conductance of the NnAChR channel in a dose-dependent manner with a K_d of 0.89 mM. The inward conductance of the channel was much less sensitive to increases of the external Mg^{2+} concentration as indicated by a K_d of 15.2 mM. The estimated maximal conductance of the channel in a divalent cation-free solution was about 52 pS for both outward and inward currents. External Ca^{2+} also reduced the inward conductance with a K_d of 6.9 mM.

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AUTORADIOGRAPHIC AND ELECTROPHYSIOLOGICAL EVIDENCE FOR $GABA_B$-RECEPTORS ON ASTROCYTES.

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By means of autoradiography we have studied the cellular localization of $GABA_A$- and $GABA_B$-binding sites in explant cultures of rat CNS. Binding of the $GABA_B$-agonist ^{3}H-(-)baclofen or of ^{3}H-GABA in presence of unlabelled bicuculline occurred to both neurones and astrocytes. Simultaneous staining of the cultures with anti-glial fibrillary acidic protein has shown that the labelled astrocytes were GFAP-positive. In contrast, binding of the $GABA_A$-agonists ^{3}H-muscimol and ^{3}H-THIP or of ^{3}H-GABA in presence of unlabelled baclofen was only observed on neurones but not on astrocytes. The lack of $GABA_A$-sites on glial cells is supported by immunohistochemical studies with the monoclonal antibody bd-17 against the $GABA_A$-receptor demonstrating that neurones are specifically stained whereas astrocytes remained unstained. From our studies it is suggested that astrocytes possess $GABA_B$-receptors but not $GABA_A$-sites. Evidence for $GABA_B$-receptors on glial cells has also been obtained from electrophysiological studies from our laboratory demonstrating that baclofen causes a hyperpolarization of cultured astrocytes.

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MODULATION OF DESENSITIZATION IN A NEURONAL NICOTINIC ACh RECEPTOR

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Desensitization of a neuronal nicotinic acetylcholine receptor reconstituted in *Xenopus* oocytes was investigated with the voltage clamp technique after nuclear injection of the cDNAs for the subunits $\alpha 4$ and non-α. The $\alpha 4$ subunit contains an intracellular domain rich in amino acid residues susceptible to phosphorylation. In order to investigate whether phosphorylation could play a modulatory role in desensitization, a hybrid brain/muscle α subunit with fewer such residues was constructed and co-injected with non-α. The resulting functional channel differed significantly from the wild type in its desensitizing properties.
In both types of receptors, desensitization during a steady exposure to ACh was biphasic and could be described with a sum of two exponentials, yielding two time constants. At -100 mV and in the presence of 50 µM ACh, the rapid phase of desensitization was slower in the hybrid (1± 0.4 s) than in the wild type receptor (0.4± 0.1 s), whereas the opposite was true for the slow phase (30± 7 s vs. 69± 19 s). Therefore, phosphorylation of the intracellular domain might modify the kinetics of desensitization. We are currently examining a receptor in which the cytoplasmic domain of the $\alpha 4$ subunit is almost completely deleted.

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STIMULATION OF SCHAFFER COLLATERALS INDUCES RELEASE OF AMINO ACIDS IN ORGANOTYPIC HIPPOCAMPAL SLICES

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A crucial criterion for establishing compounds as neurotransmitters is release following electrical stimulation of nerve pathways. Release of amino acids following electrical stimulation of Schaffer collaterals was investigated in organotypic hippocampal slices. Recordings of evoked population responses in the CA1 stratum pyramidale ensured that electrical stimulation produced synaptic transmission. Superfusate was collected via a cannula over the CA1 stratum radiatum before, during, and after stimulation, and analyzed by HPLC. Of 18 amino acids, electrical stimulation increased the release of only aspartate and glutamate, providing evidence for a neurotransmitter role of these compounds.

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METABOLIC STABILIZATION OF SYNAPTIC AChR's BY MUSCLE ACTIVITY.

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During formation of neuromuscular synapses, the nerve controls differentiation of the subsynaptic membrane via trophic effects and by induction of muscle activity. To examine the effects of activity alone on two developmental parameters, the growth of synaptic acetylcholine receptor (AChR) clusters and the metabolic AChR halflife ($t_{1/2}$), rat muscles were denervated at early steps of synapse development and kept active by exogenous stimulation. AChR number and $t_{1/2}$ were estimated from the radioactivity and its decay with time, respectively, of endplate AChR's labelled with ^{125}I-α-bungarotoxin. When developing endplates were denervated, further growth of AChR clusters was prevented and halflife of junctional AChR remained as before denervation at $t_{1/2}$= 1.8d. In contrast, muscle activity produced growth of denervated AChR clusters comparable to that observed at normally developing endplates, and stabilized the junctional AChR within less than 24hrs to $t_{1/2}$=11.5d. Given that activity reduces AChR synthesis in muscle, activity-dependent stabilization of AChR allows the growth of endplate AChR clusters in spite of reduced AChR synthesis. The experiments further show that AChR stabilization depends, besides on muscle activity, on components of the endplate membrane.

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GLUTAMATERGIC REGULATION OF GABAERGIC NEURONAL ACTIVITY

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We examined the effects of competitive and noncompetitive NMDA receptor antagonists on GABA-mediated inhibitory transmission. The administration of anticonvulsant doses of competitive NMDA antagonists did not alter steady-state levels of GABA but dose-dependently decreased both its rate of synthesis and depletion. The magnitude of these decreases correlated with the affinity of the (D,L)-antagonists for NMDA receptors, the D-isomers being more effective than the L-isomers. By contrast, noncompetitive NMDA receptor antagonists did not alter the rate of GABA synthesis, and only dizocilpine moderately decreased the rate of GABA depletion. Thus, the assessment of interactions between GLUergic and GABAergic neurotransmissions reveals differences between competitive and noncompetitive NMDA antagonists.

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NUCLEAR 3,5,3' triiodothyronine receptors (NT3R) are present in primary sensory neurons but not in Schwann cells.

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Thyroid hormones which play an important role in the development and regeneration of the nervous system require the presence of nuclear T_3 receptors (NT3R). While NT3R have been localized in nuclei of neurons and glias in rat brain, the question may be raised whether primary sensory neurons, satellite or Schwann cells, which derive from the neural crest, possess or not NT3R. Vibratome sections of DRG and sciatic nerves form newborn or adult rats were immunostained with a monoclonal antibody raised to NT3R and compared with spinal cord sections. The nuclei of all the DRG cells were strongly immunostained while all the satellite and Schwann cells were free of immunoreaction at any age. In contrast, nuclei of both neuronal and glial cells were immunoreactive in the spinal cord.
In conclusion, the immunostaining reaction allowed us to detect the presence of the NT3R in neurons of both central and peripheral nervous system but not in satellite and Schwann cells. (SNF no. 31-26410-89).

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STRIATAL NEURONS IN CULTURE - AN IN VITRO MODEL TO CHARACTERIZE NEUROTROPHIC FACTORS

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A serum-free defined culture system for dissociated neurons from embryonic rat striatum (E17-E18) has been established. Neurons were identified immunocytochemically by anti/neurofilament staining and biochemically by choline acetyltransferase (CAT) activity. The cellular composition of ten-day cultures revealed that approximately 90-95% of the cells were neurons whereas only 5% of cells represented astroglia containing glial fibrillary acidic protein. Treatment with peptide growth factors including insulin, insulin-like growth factor-I, epidermal growth factor or interleukin-1 significantly promoted in a concentration-dependent manner survival of neurons in culture but was uneffective in stimulating specific CAT activity. Under identical culture conditions as above, treatment with nerve growth factor (4nM), a neurotrophic agent for some central cholinergic neurons, markedly induced CAT activity without affecting cell viability. In conclusion, the culture system for striatal neurons may prove to be a valuable tool for characterizing neurotrophic factors.

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DO NEURONS AND GLIA DERIVE FROM THE SAME SET OF NEUROEPITHELIAL CELLS ?

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An old but still open problem in developmental neurobiology of the mammalian central nervous system (CNS) is related to two basic questions: do nerve and glial cells arise from a common cell lineage or do separate neuronal and glial precursors exist early in development and when do they appear ? An original explant culture system was elaborated, using minisegments of newborn rat optic nerves (ON), which are composed of astrocytes, progenitor cells and unmyelinated axons; neuronal cell bodies are absent. In addition to the context of developmental and functional features of glia in vitro in the absence of ganglion cells, these explants gave also rise to a neuron-like cell type, which never occurs in situ. These cells show morphological, fine structural and immunocytochemical characteristics ascribed to differentiated neurons. This observation suggests the existence of a common neuron-glia progenitor in the postnatal ON, or the presence of a small population of neuronal precursors, which never produce glia descendants in situ but is still present late in CNS development. To map the neuron-like cell lineage "downstream", we will combine the technique of retroviral gene transfer with immunocytochemical procedures. NSF 3100-009 237, Swiss MS Society.

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AN ELECTROPHYSIOLOGICAL MARKER OF THE SENSORY PHENOTYPE IN GANGLIONIC NEURONS

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The objectives of this study were (1) to determine the developmental time course of 2 membrane currents and (2) to see whether one of these currents was expressed exclusively in parasympathetic or in sensory ganglionic neurons. If so, such a current might be used as an electrophysiological marker of the parasympathetic or of the sensory phenotype.
Freshly dissociated neurons from ciliary (parasympathetic), trigeminal and dorsal root (sensory) ganglia of the quail were studied with the voltage clamp technique at various stages of the embryonic development. I_h, a cationic current activated by a hyperpolarization was present in both types of ganglia. It is expressed after embryonic day 9 (E9) and is present in more than 80% of the neurons after E14. The TTX-insensitive sodium current was observed exclusively in sensory neurons. It is expressed after E10 and is present in more than 80% of the neurons after E14. The TTX-insensitive current can be used as an electrophysiological marker and may permit to determine the phenotype of pluripotent neuronal precursors differentiating in culture.

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CYCLOHEXIMIDE PREVENTS NEURONAL DEATH DURING EMBRYOGENESIS

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We are investigating whether neuronal death during normal brain development is blocked by inhibitors of protein synthesis, such as cycloheximide (CX), focusing our work on the isthmo-optic nucleus (ION) of chick embryos. About 60% of ION neurons die between embryonic day 12 (E12) and E17.

100 µg of CX were administered to the air sac every 3 hours between E15 and E15 + 6 hours, embryos were killed at E15 + 9 hours, and paraffin sections were prepared through the ION. The number of pyknotic (dying) ION neurons was 134 ±71 in these CX-treated embryos compared with 408 ±181 in control embryos of the same age - a reduction of more than 50%. The healthy neurons were more numerous in the CX-treated embryos than in the controls. In order to determine whether protein synthesis was needed immediately prior to the onset of cell death, similar experiments were performed in embryos that had received 225ng of colchicine in one eye at E14, which blocks retrograde axonal transport and would normally lead to greatly enhanced and highly synchronized degeneration in the ION, beginning about 27 hours later. CX was administered as previously. It largely prevented the degeneration, despite being given only about three hours before it was due to begin.

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PRENATAL STRESS INFLUENCES POSTNATAL BEHAVIOR IN RATS

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Prenatal stress in rats has been shown to especially influence sex specific behaviors of the offspring. To further investigate the role of prenatal stress, pregnant rats were subjected to immobilization stress daily for 30 minutes during gestational days 15-19. Body weight of mothers and their offspring were recorded and during the first 3 postnatal months sensory and motor capacities, locomotor activity, exploration efficiency, and pain thresholds (tail flick latency) were measured in the offspring. Body weight was decreased in stressed mothers but it was increased in offspring at birth and at weaning (3 weeks old). Prenatal stress decreased locomotor activity and increased exploration efficency in male offspring when compared to the unstressed controls. In contrast, prenatally stressed female rats increased locomotor activity and decreased exploration efficiency. No differences in baseline tail flick latencies were observed; however, the analgesic effect of morphine (5mg/kg) was attenuated in prenatally stressed female rats.

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EFFECTS OF INTRAHIPPOCAMPAL INJECTION OF APV ON SPATIAL LEARNING IN THE RAT

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Systemic and intraventricular injections of NMDA (N-methyl-D-aspartate) receptor antagonists have been shown to impair learning. We have investigated the effects of intrahippocampal injection of APV (2-amino-5-phosphonovalerate) on spatial learning in a water maze using a place learning-set paradigm. Rats were trained to find an escape platform hidden 2 cm below the water surface of a pool (dia. 160cm). The platform was moved each day to one of 4 different locations; thus, the rats had to acquire a new place response daily. On test days, rats had to learn a new platform position. 15 min later, the platform was removed and the rats' search strategies during a 60-sec search test were recorded. Intrahippocamal injection of APV (50nM) before acquisition of a new platform position impaired learning, but had no effect on escape latencies to a visible platform (2cm above water surface). Animals injected with APV before the search test spent less time in the quadrant in which the platform had been. Our results support a role of NMDA receptors in spatial learning.

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DEVELOPMENT OF SEGMENTALLY HOMOLOGOUS SENSORY SYSTEMS IN THE LOCUST

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We study the development of segmental sensory systems in the locust. During embryogenesis identified clusters of sensory neurons are incorporated into a peripheral axon scaffold established by pioneer neurons. Segmentally homologous lateral cell clusters differentiate as ordered multicellular aggregates by placoid-like ectodermal invagination followed by cell migration and axonogenesis. These processes are accompanyed by the expression of the adhesive cell-cell recognition molecule Fasciclin I. In different segments these homologous clusters give rise to the wing chordotonal organ (T2-3), to the auditory organ (A1) and to chordotonal respiratory proprioceptors (A2-8). Thus, numerous sensory structures which are involved in completely different behavioral tasks are segmentally homologous and probably share a common evolutionary origin. Supported by the SNSF.

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NEUROTOXICITY & TERATOGENICITY TESTING IN VITRO: Chick Brain and Retina Cells as Monolayers, Aggregates and Reattached Aggregates.

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Mechanically dissociated brain and retina cells from 7 d old chick embryos (stage 28 according to Hamburger & Hamilton 1951) were used: (1) Cells were allowed to attach on various types of culture plates (optimally coated with or without polylysine and collagen) and used for short-term (< 1 week) toxicity testing. (2) Other cells were allowed to completely aggregate in Erlenmeyer flasks under constant gyratory movements in analogy to embryonic rat brain cells (Honegger 1985) for long-term (> 1 week) toxicity studies. Brain cells aggregate more homogeneously than retina cells, probably due to their slower aggregation mechanism at the stage of cell dissociation. (3) Aggregates of various culture age (2-20 d in vitro) were allowed to reattach to coated and uncoated culture dishes for toxicological treatment and investigation.

In all three assay systems various organic solvents were tested using routine cytotoxicity assays and critical differentiation steps as endpoints in order to evaluate the significance of these in vitro models for in vivo neurotoxicity and teratogenicity screening.

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EXPRESSION OF SUBSTANCE P AND CALBINDIN D-28k BY PRIMARY SENSORY NEURONS EVOLVES DIFFERENTLY DURING DEVELOPMENT

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The neuropeptide substance P (SP) and the calcium-binding protein calbindin D-28K (CaBP) are expressed by certain subpopulations of sensory neurons in chick DRG. During ontogenesis, SP-positive ganglion cells appear early at E5 (13%), culminate at E8 (88%), then decrease at E12 (59%) to be stabilized at 51% after hatching. In contrast CaBP-positive neurons appear later at E10 (20%) and then remain stable. To determine whether SP and CaBP were or not expressed by distinct subpopulations of ganglion cells, a double-staining based on the conjugation of a monoclonal (SP) and a polyclonal (CaBP) antibody was applied to vibratome sections of DRG. At E12, 15% of the ganglion cell bodies expressed both SP and CaBP, while only 1% displayed a double immunostaining two weeks after hatching. Since most CaBP-positive ganglion cells express simultaneously SP at E12, the two phenotypes may be colocalized at this stage. Later almost all the CaBP-positive ganglion cells lose SP-immunoreactivity so that the two phenotypes are expressed by distinct neuronal subpopulations. (SNF No. 31-26410-89)

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ASTROCYTES AND NEURONS INFLUENCE THE DEVELOPMENT OF PURIFIED OLIGODENDROCYTE PRECURSOR CELLS.

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Oligodendrocyte differentiation and myelin formation in the central nervous system is distinct for each tract and region, suggesting the existence of highly local regulatory interactions between precursors and axons or astrocytes. In culture, oligodendrocyte precursors carry specific antigens (A2B5, GD3). Factors released by astrocytes can influence the development of the precursor cells. To investigate the possible role of neurons for oligodendrocyte development we prepared a pure population of precursor cells with the help of fluorescence activated cell sorting (FACS) using the marker antibody GD3. Sorted cells were cultured in different media, conditioned media (CM), and in co-cultures. CM of sensory neurons (dorsal root ganglia) promotes survival and proliferation of the precursor cells, but clearly differs from the mitotic factors released by astrocytes (no effect of antibodies to platelet-derived growth factor). We are currently elucidating the role of direct cell contacts between neurons and precursor cells for the induction of oligodendrocyte differentiation and the expression of myelin specific markers.

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DEVELOPMENTAL RESTRICTIONS DURING AVIAN NEURAL CREST DIFFERENTIATION : A STOCHASTIC PROCESS ?

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The neural crest (NC) of embryonic vertebrates can be used as a differentiation model, since undifferentiated NC cells migrate to different locations of the embryo where they constitute the major part of the peripheral nervous system, the melanocytes of the body and many skeletal and connective tissues constituting the head. A recent analysis of the developmental potentialities of individual quail mesencephalic NC cells, performed in vitro, has revealed several types of progenitors. Multipotent progenitors, giving rise to all kinds of derivatives, monopotent ones, differentiating to only one or the other of these derivatives, and almost all possible intermediate oligopotent stages were observed. The variety of progenitors found in this study suggests a differentiation model analogous to the hematopoietic system, i.e. through progressive and stochastic restrictions in cell lineage.
We have analyzed by Factorial Correspondance Analysis 450 clones obtained from single mesencephalic NC cells cultured in identical conditions. The phenotypic composition of these clones is consistent with a stochastic restriction model.

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DIFFERENTIAL RESPONSES OF ASTROCYTES TO EPIDERMAL GROWTH FACTOR IN TELENCEPHALON CELL AGGREGATE CULTURES. DEPENDENCE ON THE DEVELOPMENTAL STAGE.

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Using serum-free aggregating cell cultures of fetal rat telencephalon we could show previously that during the first week *in vitro*, when most glial cells are still dividing, epidermal growth factor (EGF) given at low doses (5 ng/ml) greatly enhanced the differentiation of glioblasts (precursors of astrocytes and oligodendrocytes). At high doses (90 ng/ml), EGF produced an additional mitogenic effect. We have now examined the effect of EGF in postmitotic cultures. We have found that continual treatment of the cultures with high doses of EGF between days 18 and 30 *in vitro* induced mitotic activity of astrocytes but not of oligodendrocytes. A large proportion of astrocytes were responsive already two days after the first addition of EGF. The activity of glutamine synthetase, an astrocyte-specific marker enzyme, decreased with increasing mitotic activity. These results demonstrate that astrocytes already engaged in terminal differentiation are able to respond to EGF by re-entering the mitotic cycle, and that this response is clearly distinct from that of mitotically active astrocyte precursors. Supported by the SNF, Grant 3.601.87.

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DEVELOPMENTAL EXPRESSION AND EVOLUTIONARY CONSERVATION OF SNAP-25

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First described in the mouse, Synaptosomal Associated Protein-25 (SNAP-25), is a cytoplasmic protein mainly found in axons and terminals, and is thought to play a role in synapse function via its association with vesicles and presynaptic membranes (Oyler et al. submitted). Chick cDNA clones encoding SNAP-25 were obtained by screening a cDNA library from embryonic day 15 chick retinas, with a probe to the coding region of the mouse SNAP-25. The sequences of the chick cDNAs show 92% nucleotide and 100% amino acid sequence identity to the mouse. Motor and spinal ganglia neurons of the spinal cord, as well as amacrine and ganglion cells of the retina, express high levels of SNAP-25. Expression begins late during neuronal differentiation, when neurons establish contacts with their targets, and make synapses.

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THE ISOFORMS OF TENASCIN AND THEIR DIFFERENTIAL EXPRESSION DURING CORNEAL DEVELOPMENT.

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Tenascin is a large extracellualr matrix glycoprotein consisting of either 190 kDa or 200 kDa or 220 kDa polypeptide chains assembled into hexameric structures. Affinity chromatography using a monoclonal antibody T17, specific for Ten220, separates tenascin into two populations, Ten220 and Ten200/190. The separation of tenascin into homohexamers composed of either Ten220 or Ten200/190 now permits examination of isoform specific functions. In electron micrographs the T17 epitope is localised 2/3 along the arm of the hexabrachion, corresponding to the splicing in or out of three type III homology domains (Jones, F.S., et al. (1989) PNAS 86:1905). The distribution of tenascin isoforms is spatially and temporally regulated during development. For instance, Ten220 is not detected in 13d to 19d chicken embryo sterna, whereas in brain at the same stages only Ten220 and Ten190 occur. In the chicken cornea, T16 labels the endothelium throughout embryonic development. At later stages T16 detects tenascin in the stroma (39–45) in addition to the Bowman's membrane (30–45). T17 only labels the endothelium at stages 22–24. Neither stroma nor epithelium contain Ten220. The occurrance of Ten220 in the cornea seems to be restricted temporally and spatially to neural crest cell pathways.

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TENASCIN EXPRESSION AND FUNCTION DURING PNS DEVELOPMENT

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The extracellular matrix protein, tenascin, is expressed in a restricted spatial and temporal pattern in the embryo. During formation of the peripheral nervous system of the chick, it is accumulated within sensory and sympathetic ganglia and along axon fascicles of the ventral roots. The first tenascin to be detected in the limb buds is associated with the ingrowing motor nerves. To probe its function, we investigated the effects of a tenascin substrate on cultured nervous tissue explants and single neurons obtained from 6-11d chick embryos. Single sympathetic and sensory neurons did not attach to pure tenascin substrates, but attached and formed neurites on polylysine/tenascin. Outgrowth started after 6h at about half the rate observed on laminin, and could be inhibited by antibodies against tenascin and β_1-integrin. In contrast to peripheral neurons, spinal cord neurons started to sprout immediately after plating onto polylysine/tenascin. Pure tenascin substrates sustained neurite outgrowth from nervous tissue explants. While the elongating neurites attached poorly and tended to fasciculate, they had well-spread, highly motile growth cones at their tips. Thus, tenascin could affect growth cones and neurites differently.

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EMBRYONIC MOTONEURONS GRAFTED INTO THE ADULT CNS CAN DIFFERENTIATE AND MIGRATE

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Grafting of embryonic neuronal tissue into the CNS of a mammalian host is now considered to be an established experimental technique. In most studies, a heterogeneous population of cells has been transplanted due to the difficulty of dissecting out a particular class of central neurons. Here we describe experiments in which an identified neuronal cell population has been grafted into the adult CNS. The technique involves the specific labelling of embryonic spinal motoneurons by retrograde transport of a fluorescent tracer followed by purification of the dissociated motoneurons on a density gradient and cell-sorting. The fluorescently-labelled embryonic mouse motoneurons were grafted into the spinal cord (a natural site) and the striatal region of the brain (an ectopic site) of adult mice; these neurons were found to survive for at least 10 weeks as judged by their round, bright appearance and to differentiate as assessed by their ability to extend axons and/or dendrites. It was surprising that these motoneurons could migrate long distances in the spinal cord (2 mm) and in the brain (4 mm) and that they could survive in both the grey and white matter.

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RAT BRAIN AROMATASE AFTER PRENATAL NICOTINE EXPOSURE

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Previous investigations in our laboratory showed persistent effects of nicotine on central monoaminergic systems and male sexual behavior in rat fetus and offspring. In this study, we determined the effect of prolonged nicotine treatment during gestation on brain steroid aromatase which converts androgens to estrogens and is known to be important in sexual brain differentiation. Enzyme activity was determined by the conversion of 1ß-^{3}H-androstenedione to estrone (a modification of Thompson & Siiteri). At an early postnatal stage, aromatase activity in a brain region comprising preoptic, hypothalamic and amygdaloid areas was reduced in prenatally treated male offspring but unaffected in females. These results indicate a selective effect of the drug on aromatase in males.

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EFFECT OF PRENATAL NICOTINE EXPOSURE ON CENTRAL NICOTINIC BINDING SITES AT DIFFERENT STAGES OF DEVELOPMENT

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Time pregnant Long Evans rats were treated with nicotine delivered by subcutaneously implanted Alzet minipumps from gestational day (GD) 12-19. 3H-nicotine binding sites were studied in brain of offspring by quantitative in vitro autoradiography (Odermatt et al. Experientia 43 (87)), using a modified ASBA image analysis system.
At GD 21, offspring of nicotine-treated dams showed a high increase in the numbers of binding sites in several brain regions. The density of binding sites remained elevated in neocortical layers III/IV until adulthood, whereas caudate-putamen and substantia nigra pars compacta showed a decrease below control level during that period. The laterodorsal thalamic nuclei, the lateral geniculate nucleus and the central gray of the pontine area were at normal levels after birth.
These results indicate that the immediate effect of prenatal nicotine is similar in different brain regions, whereas the secondary changes occuring after termination of treatment differ between regions.

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NEUROIMMUNE EFFECTS OF PRENATAL DIAZEPAM EXPOSURE

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Low doses of diazepam (1.25 mg/kg daily) given to pregnant Long Evans rat dams from gestational day 14 to 20 result in longterm depression of cellular immune functions in male and female offspring (Schlumpf et al., Life Sci., 44, 493, 1989). These changes may involve peripheral and/or central drug actions. In view of a possible involvement of the autonomic nervous system, we investigated the sympathetic innervation of the thymus. Norepinephrine levels (determined by HPLC-EC) were elevated at postnatal day 7 in both sexes and day 22 in males, i.e., during the time period of cellular immune depression. Studies of Con A-stimulated T lymphocyte proliferation in vitro also show direct effects of benzodiazepine agonists and antagonists. They are presently being analyzed with respect to the sensitivity of different cell types (T lymphocytes and adherent cells).

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EARLY NEURON PATTERN IN THE DEVELOPING ANTENNAL DISC OF *DROSOPHILA* PUPAE.

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We have studied the early pattern of neurogenesis in the antennal disc of D. melanogaster by applying a monoclonal antibody *(22C10)* to cryosections of pupae or antennal whole mounts. This antibody binds to an antigen which is expressed during early stages of neuronal differentiation until the adult stage. No neurons are stained in the antennal disc of wandering third instar larvae or white pupae; this is in contrast to the eye or leg discs. Neuronal cell bodies become visible in the antennal disc only 3 hr after puparium formation. These early neurons are located in the center of the disc which will give rise to the distalmost structure of the antenna, i.e., the arista. Hence, these neurons, which may be precursors of the aristal sense organ, are likely candidates of antennal pioneer neurons. Between 3 to 8 hr after puparium formation, another 6 clusters of neurons are formed. By following their maturation during metamorphosis, we were able to determine their fates in the adult antenna, i.e., whether they will give rise to olfactory sensilla on the funiculus or form mechanoreceptors in the Johnston's organ.

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GLUTAMATE IMMUNOREACTIVITY IN THE DEVELOPING RAT CEREBELLUM

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Glutamate is believed to play a major role as excitatory neurotransmitter in the adult cerebellar cortex. We have investigated the pattern of glutamate-like immunoreactivity in the developing cerebellum of early postnatal rats. Parallel fibers are glutamate-immunoreactive already in the early postnatal period. Around the perikarya of Purkinje cells, some immunoreactive dots were noticed which seemed to correspond to climbing fibers and their terminals. In the granule cell layer, large terminal-like profiles were found to resemble mossy fibers endings. The three major excitatory systems in the rat cerebellar cortex, thus, contain glutamate-like immunoreactivity already in their early development.

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COMPARISON OF RESULTS OBTAINED BIOCHEMICALLY AND BY ^{1}H MAGNETIC RESONANCE SPECTROSCOPY OF THE RAT BRAIN DURING DEVELOPMENT

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Data obtained by a new method such as ^{1}H magnetic resonance spectroscopy (MRS) have to be compared with biochemical data. Therefore, we compared in vitro ^{1}H MRS measurements of rat brain extracts (rats: 2-59 days old) with biochemical measurements and in a next step also with preliminary results of in vivo MRS. The following substances can be reliably measured in brain extracts by in vitro MRS : N-acetylaspartate (NAA), total creatine (Cr), phosphorylethanolamine (PE), Tau, Glu, Gln, GABA, Ala, lactate and inositol. We show a comparison of different methods of MRS data evaluation and biochemical data on the first 8 substances. During development of the rat from day 2-59 concentrations of PE, Tau and Ala decrease, those of NAA, Cr, Glu and Gln increase, while GABA does not change. The developmental pattern of these substances is the same, whether obtained by in vitro MRS or biochemical methods. Quantitation of NAA, Cr, Tau, GABA and PE leads to the same results with both methods, while Glu, Gln and Ala concentrations determined by in vitro MRS are lower than measured biochemically. The ratio of NAA/Cr in the rat brain during development was also determined by in vivo MRS. These preliminary results correlate with biochemical and in vitro data.

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MOLECULAR GENETICS OF A MAJOR SWITCH IN *DROSOPHILA* DEVELOPMENT

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We show that DAPA is a novel external cell surface protein encoded by a gene located at 100B1,2 and induced by the ecdysone pulse signaling the major switch from proliferation to differentiation of the imaginal cells during the late third instar period. Our evidence indicates that DAPA interacts with the product of a neighboring gene, *l(3)dco*, to trigger the mitotic arrest preceding differentiation.- DAPA was isolated as a 15S glycoprotein complex by methods developed earlier by one of us (H.N.) for sea urchin embryos. A cDNA clone isolated with anti-DAPA antiserum predicts a cysteine-rich secreted 954 amino acid glycoprotein corresponding to the 120 kd subunit of the complex and identical with the partial sequence of a gene encoding a cell adhesion protein induced by ecdysone in S3 cells (W. Rickoll, pers. comm.). The DAPA gene was shown to be different from Bryant's lethal disc overgrowth mutant gene l(3)dco. However, anti-DAPA mAbs staining the surface of wild-type disc cells are not bound to the surface of l(3)dco discs, presumably because of lack of a functional transmembrane receptor.

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ISOLATION OF A *PRD*-TYPE HOMEOBOX GENE CLUSTER INVOLVED IN *DROSOPHILA* MORPHOGENESIS

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Three new *prd*-type homeobox genes (PPH13;PPH25;L13-4) located within the chromosomal region 21C1/21D1,2 of *Drosophila* have been isolated. PPH13 and PPH25 are closely linked and correlate with the *dachsous* (*ds*) locus. The third homeobox resides more upstream of *ds* within a region that is deleted in *Df(2L)al*. *ds* belongs to a group of "leg genes" (*ds, d, fj, cg*) showing similar mutant phenotypes mainly affecting legs and wings. Combinations of these mutations exhibit impressive qualitative changes in histogenesis (homeotic transformations (Waddington,1943)). We present our latest results concerning the molecular characterization of these genes. The cloning of the genomic DNA defining the *ds* locus, the localization of various chromosomal rearrangement breakpoints and a time course of the transcriptional profile will be shown. We also present a Northern analysis as well as the transcript distribution in the wild-type embryo of the L13-4 homeobox gene.

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SODIUM AND CHLORIDE TRANSPORTS IN THE EARLY CHICK EMBRYO

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Transblastodermic short circuit current (Isc), open circuit potential (Voc) and conductance (Gtot) were measured in the in vitro gastrulating chick embryo. Simultaneously, fluxes of ^{22}Na (J_{Na}) and ^{36}Cl (J_{Cl}) in dorsal-ventral (DV) and opposite (VD) directions were determined. Steady state values were : Isc = 19.6 (± 4.3) μAcm^{-2}, Voc = 14.0 (± 1.5) mV and Gtot = 1.8 (± 0.6) $mScm^{-2}$. Chloride flux was purely passive (proportional to Gtot) and equal in DV and VD directions : 1.97 $\mu Eq\ hr^{-1}cm^{-2}$. J_{Na}-DV was by 64% higher than the value predicted by Isc. J_{Na}-VD was zero for Gtot < 1.6 $mScm^{-2}$ and then linearly related to Gtot (0.07 $\mu Eq\ hr^{-1}\ cm^{-2}$ at Gtot = 1.8 $mScm^{-2}$). With ouabain (10^{-4} M) at the ventral side Isc decreased by 83%, whilst J_{Na} in the DV direction decreased only by 48%. These results show a net DV transport of sodium, which accounts for Isc and an asymmetrical diffusion of sodium. This asymmetry suggests that the blastoderm is not a simple epithelial layer, but that an additional compartment must be taken into account (i.e. the extracellular intraembryonic space, the formation of which is linked to the ionic transport).

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SUPRACELLULAR ORGANIZATION OF EXTRACELLULAR MATRIX IN THE CHICK EMBRYONIC EPITHELIA

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It is known that the organization of the extracellular matrix (ECM) modulates the behaviour of adjacent cells (e.g., shaping, migration) but it is not known how the ECM itself becomes organized. The chick embryo is an excellent system allowing to study this problem.

The ectoderm of one-day chick blastoderm consists of a pseudo-stratified epithelium in the embryonic area pellucida and a monolayer of polygonal cells in the extraembryonic area opaca. Both epithelial layers undergo during the earliest embryogenesis a constant remodelling and a characteristic spatial and temporal differentiation in their metabolism, transports, motility and morphology (J.Cell Biol. S8:415,1987). They also produce ECM assembled in organized networks depending on developmental stage and region of the blastoderm (Cell Tiss.Res. 241:92,1985).

The comparison between the ECM patterns and gradients of cell functions (Biol.Bull.S176:118,1989) suggests two factors that might modulate the supracellular organization of ECM, i.e., mechanical tensions generated by embryonic cells, and, ionic currents flowing through the pericellular space into which the ECM is secreted.

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Na,K-ATPase EXPRESSION DURING XENOPUS DEVELOPMENT

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In Xenopus laevis (X.l.), regulation of Na^+ transport via the Na,K-ATPase (NKA) plays an important role in distinct developmental programs. In this study, we have examined how NKA activity evolves after fertilization of X.l. eggs up to neurula formation and what are the mechanisms of its regulation. NKA activity remains low during early cleavage stages, doubles at midblastula and again at neurula stage. The rise in NKA activity is accompanied by an increased biosynthesis of catalytic α-subunits leading to a 20 to 50 fold cellular accumulation of two α-isoforms as detected on immunoblots. In contrast, the glycoprotein ß-subunit of NKA cannot be detected until late neurula. These data indicate that during early X.l. development 1) the NKA activity is modulated in parallel with functional needs namely during blastocoele and neurula formation 2) the expression of ß-subunits limits the number of functional NKA and 3) α- and possibly ß-isoforms are expressed in a developmentally regulated fashion.

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EVIDENCE THAT ACIDIFICATION OF THE YOLK SPHERES PROMOTES YOLK DIGESTION IN TICK EGGS THROUGH ACTIVATION OF A LATENT PROCATHEPSIN L.

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Tick eggs are filled with yolk spheres that contain typical lysosomal acid hydrolases. A cathepsin L-like cysteine proteinase has been characterized in detail. It is latent when tested under mildly acidic conditions, but activated by previous treatment at lower pHs. When localized through their gelatinolytic activity on gelatine containing SDS-PAGE, latent and active forms display different band patterns, strongly suggesting that cathepsin L is stored as a proenzyme which is processed to the mature, active form both in vitro, at low pH, and in vivo, concomitantly with yolk degradation. We investigated the pH of the yolk spheres with acridine orange. In early development all spheres are neutral, but then a progressively increasing number acidify, in parallel with procathepsin processing and yolk digestion. Dense yolk spheres separated on a Percoll gradient are neutral and contain solely the proenzyme, while acidic spheres and mature cathepsin cosediment in fractions of lower density. Thus acidification appears to be the regulatory key that promotes activation of cathepsin L, which in turn is responsible for yolk hydrolysis.

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DEVELOPMENTAL REGULATION OF Na, K-ATPase ISOFORMS DURING EARLY DEVELOPMENT IN XENOPUS LAEVIS.

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Active sodium transport across the plasma membrane mediated by the Na, K-ATPase appears to play a critical role during oocyte maturation, egg fertilization and early development in Xenopus laevis. Na, K-ATPase is a plasma membrane protein composed of 2 subunits α and β. In differentiated kidney cells, α and β subunits are synthesized in a steochiometric 1 to 1 and coordinated manner. By contrast at stage VI oocyte, we observe a much lower rate of translation of the β compared to the α subunit.

We have analysed the expression of the α and β subunits of the Na, K-ATPase at the mRNA level from fertilization up to neurula, by northern blot analysis and by primer extension technics; we can demonstrate that at stage VI oocyte there is a vast excess of α mRNA over β mRNA, excluding the hypothesis of a large untranslatable β mRNA pool. During the early development the β mRNA level remains much lower than the α mRNA level confirming the hypothesis that the β mRNA is the limiting factor in the assembly of a functional Na, K-ATPase. The analysis of a new β isoform (β2) changes the ratio α mRNA over β mRNA during the early development. In addition We have obtained indirect evidence for the presence of a new fetal form of the Na, K-ATPase α subunit that we are currently cloning.

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PROTEIN KINASE C ACTIVITY IS NOT RESPONSIBLE FOR THE EXPRESSION OF LONG-TERM POTENTIATION IN HIPPOCAMPUS

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Long-term potentiation (LTP) in hippocampus, an increase in synaptic efficacy induced by high frequency stimulation, has been proposed to result from a tonic activation of protein kinase C (PKC). This possibility would require stimulation or inhibition of the kinase to affect differentially synaptic responses generated on control and potentiated pathways. Specifically activation of PKC should produce a smaller facilitatory effect on previously potentiated inputs and conversely blockade of the enzyme should eliminate the LTP effect. We tested these predictions using phorbol esters to activate and H-7 to inhibit PKC and found that the actions of the drugs were not affected by prior induction of LTP. Phorbol esters enhanced and H-7 reduced to the same degree control and potentiated responses. Both compounds however significantly decreased the contribution of N-methyl-D-aspartate receptors to synaptic potentials, a result that accounts for their suppressive effect on LTP formation. Thus, PKC is probably not involved in the expression of LTP but may play an important role in its induction. Work supported by FNRS 3.173.0.88.

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