Symposia

S1 1

SIGNAL RECOGNITION IN PROTEIN TRANSLOCATION ACROSS THE ENDOPLASMIC RETICULUM MEMBRANE

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Hydrophobic signal sequences are known to direct the translocation of proteins across the endoplasmic reticulum (ER) membrane. We have used a cross-linking approach to identify protein receptors which recognize these signals. A photoreactive group is attached to the ε-amino group of lysine in charged Lys-tRNA. The altered lysine is still accepted by the translational apparatus and can be incorporated into the signal sequence of preprolactin. After irradiation, interacting components can be identified. Based on this approach two receptors have been identified: 1) a polypeptide component of the signal recognition particle (SRP) (M_r 54,000), and 2) a signal sequence receptor (SSR) in the ER membrane which is an integral, glycosylated component (M_r 35,000). SSR is probably the basic targeting device whereas SRP is needed for most proteins to prevent their aberrant folding before translocation.

S1 2

AMINOACYLATION OF tRNAs WITH L-4'-(3-(TRIFLUOROMETHYL)-3H-DIAZIRIN-3-YL)PHENYLALANINE

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L-4'-(3-(Trifluoromethyl)-3H-diazirin-3-yl)phenylalanine ((Tmd)Phe) is a photoactivatable, carbene-generating analogue of L-Phe. Towards our goal of incorporating, by in vitro translation, this amino acid into proteins, (mis)aminoacylation of tRNA(s) has first to be achieved. Two procedures were found to afford the mischarged tRNA(s). The first one, based on work of Hecht and collegues, involved T4 RNA ligase-mediated coupling of chemically prepared aminoacyl-pCpA with E. coli tRNA or E. coli $tRNA^{Phe}$ missing at the acceptor stem the last two nucleotides (pCpA).
Mischarging was also accomplished enzymatically of E. coli $tRNA^{Phe}$ using a heterologous system that included phenylalanyl-tRNA synthetase from yeast (S. cerevisiae), (Tmd)Phe (4 mM) and 20% DMSO. Characterization of the "chemically" and enzymatically mischarged tRNA(s) was done (i) by chemical deacylation followed by enzymatic reaminoacylation with L-Phe and (ii) by measurements of electrophoretic and chromatographic behaviours of the tRNA species.

S1 3

MODIFIED PRECURSOR PROTEINS AS TOOLS TO STUDY THE TRANSPORT OF PROTEINS INTO MITOCHONDRIA

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It is known that presequences direct precursor proteins to the correct cellular organelles but the mechanism of the transmembrane movement of a precursor protein is still not understood.To study this process we used an artificial mitochondrial precursor protein containing a mitochondrial presequence fused to mouse dihydrofolate reductase (DHFR) (Hurt et al. EMBO J. 3, 3149, 1987).We modified this protein by oligonucleotide directed mutagenesis to address the questions: 1)What happens to the tertiary structure of the precursor during translocation? 2) Could the precursor be stopped at a late stage of the translocation process by a chemical modification at its C-terminus and could this transport intermediate be used to identify components of the translocation machinery? We found that point-mutations which destabilized the precursor protein dramatically enhanced its posttranslational import into isolated mitochondria. This strongly indicates that the protein must unfold to enter mitochondria. We are currently testing the import of a modified precursor containing at its C-terminus a cysteine residue derivatized with biotin. This should allow us to test if the addition of avidin to the import reaction generates a translocation intermediate.

S1 4

NUCLEAR PROTEIN LOCALIZATION IN YEAST

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We are studying 1) the signals within a nuclear protein which are responsible for its nuclear localization and 2) nuclear envelope components with which these signals presumably interact. The yeast repressor α2 has been shown to have two distinct nuclear localization signals. Both signals are necessary for targeting α2 to the nucleus. One signal is at the amino terminus of α2; the second signal is in a central portion of α2 which is homologous to the homeo-domain. Differences between the two signals suggest that they are not functionally equivalent and that they might act at different steps in a localization pathway. A lamin-like protein has been purified to near-homogeneity from isolated yeast nuclei. This protein forms a macromolecular network of ~10 nm filaments which resembles the nuclear lamina isolated from other organisms. This network contains occasional "donut"-like ring structures at sites where filaments converge. The ring structures are similar in size to nuclear pore complexes but do not have the octagonal symmetry typical of nuclear pores.

S2 5

MOLECULAR PROPERTIES AND FUNCTIONAL SIGNIFICANCE OF THE NICOTINIC ACETYLCHOLINE RECEPTOR-CHANNEL.

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It will be a challenge for the coming years to relate receptor structure to function. We have found that adult frog muscle nicotinic receptors behave as a homogenous population. All agonists that have been tested (eg. acetylcholine, suberyldicholine, carbachol and suxamethonium) can block ion channels but, apart from this action, are effective 'full agonists'. Differences in potency between them result more from differences in binding affinities than from differences in ability to open the channel once bound. The nicotinic receptor channel in dissociated sympathetic neurones differs in many ways from that in muscle. Antagonists that are primarily competitive in muscle work by channel block in neurones; the neuronal channel shows very strong inward rectification (regardless of the nature of the intracellular ion), and the channels appear to be heterogenous. It is hoped that expression of cloned receptor subunits in oocytes will clarify the complexities of the neuronal receptor-channel.

S2 6

REGULATION OF ION CHANNELS IN EXCITABLE ENDOCRINE CELLS

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Ion channel function in endocrine cells is controlled by hormones and neurotransmittors. These factors are recognized by cell surface receptors, which in turn affect channel function either directly or indirectly i.e. via intracellular second messenger. To study this regulation, we have monitored cytosolic $[Ca^{2+}]$ by microfluorimetry with fura 2 and ion channel function simultaneously, in the pituitary cell line GH_3B_6, using the patch clamp technique in its whole cell configuration. With this approach it was demonstrated that excitability, i.e. action potential firing, of pituitary cells provides a means for cell activation (Schlegel et al. 1987, Nature 329, 719). Furthermore, the approach yields a refined analysis of the activation of K^+ currents by Ca^{2+} mobilizing hormones and of the role of modulators of Ca^{2+} channels for the Ca^{2+} entry during the action potential.

S2 7

Ca CHANNELS AND NEUROTRANSMITTER RELEASE

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Entry of Ca, Ba and Sr into presynaptic nerve terminals has been correlated with the release of serotonin (5-HT). Pairs of identified neurons dissected from the leech CNS form synapses without profuse outgrowth in a few days on polylysine in L-15 medium. The presynaptic neuron is the Retzius cell (known to secrete 5-HT) and the postsynaptic cell is a sensory P neuron. These cells are isopotential and under voltage clamp, with Na and K currents blocked, depolarization of the presynaptic Retzius cell gives rise to an inward current carried by Ca, Ba or Sr followed by release of 5-HT. The synaptic potentials are steeply dependent on the amplitude and duration of the divalent cation currents and on facilitation. Similar results are obtained by measuring Ca transients optically with Arsenazo III. With this technique, as with loose patch clamp, one can record Ca entry over different regions of the neuronal surface, such as soma, initial segment and growth cones.

S2 8

DIFFERENTIAL REGULATION BY PROTEIN KINASE C OF FOREIGN NEURONAL ION CHANNELS EXPRESSED IN XENOPUS OOCYTES

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Total mRNA was isolated from 2d-old chick brain and injected into Xenopus oocytes. This lead to the formation in the oocyte plasmamembrane of ion channels typical for neurones. Channel currents could be measured by using electrophysiological techniques. We investigated the voltage gated Na and Ca-channels (N-type) and the ligand-gated GABA and kainate channels. Upon exposure of the oocytes to 5 nM ß-phorbol myristate acetate (ß-TPA) the peak current amplitudes of the Na and the GABA channel showed an exponential decrease with a half-time of about 15 min. In contrast no effect could be observed on the kainate channel, but the amplitude of the Ca-current increased to about 180% of the control amplitude within 15min. Control application for 30min of 100nM of either α-phorbol or α-TPA were without effect on all current amplitudes. Tamoxifen (100nM) prevented the effect of ß-TPA. 100µM oleoylacetylglycerol was also active in altering the ion currents, but the effects were transient and smaller than those seen with ß-TPA. These observations suggest a differential regulation by protein kinase C of ion channels involved in neural transmission.

S3 9

MOLECULAR-PHARMACOLOGICAL ASPECTS OF THE MODE OF ACTION OF ANTIDEPRESSANTS

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Clinically effective antidepressant treatments cause a delayed desensitization of the noradrenaline (NA) beta adrenoceptor coupled adenylate cyclase system in brain linked to a down-regulation of beta adrenoceptors. Non-linear regression analysis of agonist competition binding curves reveals that the reduction in the density of beta adrenoceptors following antidepressants is confined to the receptor population displaying high agonist affinity. Na regulates the population of beta adrenoceptors in the high and serotonin (5HT) in the low agonist affinity conformation. Glucocorticoids represent the third physiologically important group of regulators mediated via alpha$_1$ adrenoceptors. The final common pathway of signal transduction seems to be protein kinase mediated phosphorylation via second messengers derived from NA/5HT receptor interactions. The demonstration of beta adrenoceptor mediated changes in gene expression raises questions on stimulus-transcription coupling and adds a molecular perspective to the "5HT/NA link hypothesis" of affective disorders and their pharmacotherapy.

S3 10

ETHOLOGICAL ASPECTS OF ANTIDEPRESSANT ACTION

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Behavioural actions of antidepressants are limited to antagonism of various forms of immobility e.g. terabenazine-induced catalepsy, immobile response to swimming, and facilitation of positively reinforced brain stimulation. In contrast to these non-social situations, antidepressants have been reported to "stabilize" social behaviour in rodents and to alter social interactions in primates. A common factor of these different studies appears to be "the release" from a blocked behavioural state and/or the attenuation of defensive patterns of behaviour. In rodents, some antidepressants appear to facilitate the release of social behaviour and in mice assist the pursuit of a socially dominant mode. Ethologically, depressed patients appear to exhibit a lack of searching behaviour, a lack of approach-oriented components which hamper normal social functioning. It is suggested that effective antidepressants should re-instate social behaviour either by promoting responses to relevant social stimuli and/or attenuating blocked escape reactions.

S3 11

PHARMACOLOGY OF ANTIDEPRESSANTS (AD): CLINICAL ASPECTS

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Methodological shortcomings may be responsible for the fact, that despite clear cut differences in the biochemical action of AD so far no relevant clinical differences between the various drugs have been found. So far, no generally accepted biological marker is available to monitor effects of AD drugs. Therefore, therapeutic effects are assessed by either self-reported or observed changes in the patient's mood and behaviour. Both methods rely on subjective judgement rather than on objective measurement. Observation of complex behaviour is difficult and reliability rather low. There are, however, two methods which might improve the quality of the clinicians' observation.
1. Improvement of the physicians' consistency by the computer aided judgment analysis.
2. To break down the complex behaviour in ethologically relevant elements which can be assessed by observer-independent methods. Because of the limited therapeutic effects of (AD) and the fluctuations in the natural history of depression, the assessment of drug effects needs carefully designed placebo controlled studies.

S4 12

GENETIC POLYMORPHISMS OF DRUG OXIDATION. PHARMACOLOGICAL, BIOCHEMICAL AND IMMUNOLOGICAL ASPECTS

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Several genetic polymorphisms in the oxidative metabolism have been recently identified. That of debrisoquine oxidation has been extensively studied. Two phenotypes have been observed: most subjects are extensive metabolizers (EM) whereas a minority of subjects with an impaired debrisoquine oxidation are poor metabolizers (PM). The PM phenotype is inherited as an autosomal recessive trait and its prevalence ranges from 3 to 10% in Europe. Now, more than 30 drugs are known to be oxidized by the same metabolic pathway and to display an oxidation polymorphism co-segregating with that of debrisoquine. Similarly other types of drug oxidation polymorphism have been observed for mephenytoin, tolbutamide and nifedipine. The biochemical mechanism of these polymorphisms is related to a functional deficiency or a decreased amount in some isozymes of hepatic cytochrome P-450 recently characterized. Interestingly some of these isozymes may be the target-proteins of serum antibodies (anti-liver kidney microsomes), present in some autoimmune diseases.

S4 13

GENETIC POLYMORPHISMS AND VARIATIONS IN DRUG REACTIONS

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The two most classical genetic polymorphisms in drug biotransformation are those of N-acetylation and debrisoquine-type oxidation. In "poor metabolizers", deficient first-pass metabolism after oral dosing may cause early drug toxicity (debrisoquine, bufuralol, sparteine) or lack of bioactivation (codeine); low metabolic clearance accounts for toxic cumulation in long term therapy (perhexiline, tricyclic antidepressants, sulphasalazine, hydralazine, procainamide) and alternative metabolite production is responsible for unusual toxicity (phenacetin). The most spectacular impact of these genetically controlled variations are certainly drug interactions involving compounds capable of, for instance, "transforming" extensive metabolizers in phenotypically poor metabolizers (quinidine, neuroleptics). Phenotypes thus have a dramatic impact on kinetics variations, but links with the occurrence of side-effects are less documented. The development of easy to perform phenotyping procedures should still aid in specifying the true clinical impact of these phenotypes. Human liver banks now allows development of in vitro models which show predictive value for in vivo behavior.

S4 14

THE ASSESSMENT OF POLYMORPHIC DRUG ACETYLATION AND ITS CLINICAL CONSEQUENCES

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Since its discovery 30 years ago, the genetic polymorphism in human liver N-acetyltransferase has been shown to affect the disposition of many useful drugs as well as some suspected amine carcinogens. This often leads to an increased incidence of adverse reactions in individuals who are either unable to efficiently eliminate potentially toxic parent compounds or who produce elevated levels of toxic metabolites. Acetylator phenotype has also been associated with certain other apparently spontaneous disorders of unknown etiology. It can therefore be useful to determine acetylator status, and various methods have been developed for this purpose using test drugs which are polymorphically acetylated. Of these, the sulfamethazine and dapsone tests have been most widely applied, but a newer test using caffeine eliminates many of the problems associated with the older methods. In the future, in vitro phenotyping tests using antibody or gene probes could be applied, although in light of recent findings suggesting complex molecular mechanisms for this and other polymorphisms, in vivo tests may remain for now the more practical approach for the clinician.

S4 15

STRUCTURAL FACTORS CONTROLLING THE POLYMORPHIC METABOLIZATION OF DRUGS: COMPUTATIONAL APPROACHES

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Computational methods are being used to study drug-receptor interactions in debrisoquine/spartein (D/S) polymorphic cytochrome P450 drug oxidation. The goals of this work are first, to obtain additional insight into the mode(s) of action of polymorphic cytochrome P450, and, second, to find structural parameters (reactivity indices) which may help identify those drugs which are polymorphically metabolized by the D/S isoenzyme. In this paper we present results of our initial studies on substances which inhibit or are oxidized by this isoenzyme. Two aspects are considered: First, conformational energy studies on substances with measured inhibition constants and model building of these compounds to the rigid substrate, dextromethorphan, and second, the search for and analysis of electronic properties which may be reactivity indices for complex formation with the D/S cytochrome P450 binding site.

S5 16

CA RELEASE IN SKELETAL MUSCLE AND ITS CONTROL BY THE MEMBRANE POTENTIAL

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The primary signal that controls Ca release from the sarcoplasmic reticulum (SR) in skeletal muscle is the potential difference across the cell membrane. Three functional units of the system can be postulated: 1. a voltage sensing element in the transverse tubular invaginations of the cell membrane, 2. a Ca channel in the adjacent SR membrane, and 3. a transmitting element between both membranes. Signals originating from two of the three units have been studied simultaneously in experiments on isolated segments of single muscle fibres: 1. Information about the voltage sensor can be obtained by applying voltage steps to the cell membrane and analysing the current signals which result from an intramembrane charge displacement. 2. Ca release can be quantified by using a sensitive and rapid Ca probe to measure Ca transients in the sarcoplasm. In addition to the rapid movement of sensor charges in response to voltage steps a slow adaptation of the Ca release system can be observed at steady membrane polarization. This process is accompanied by an alteration of the voltage dependence of the intramembrane charge movements and can be interpreted by assuming a transition of the voltage sensor between an active and an inactive operating mode.

S5 17

ENERGETICAL CONSIDERATIONS ABOUT CA^{++}-RELEASE IN SKELTAL MUSCLE

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Activation of 1 g of muscle requires release of about 200 nmol of Ca^{++} from the SR. If this lasts 5 ms a current of about 8 A results. Since at least 90% of Ca^{++} is released at a gradient below 1000/1 a free energy of about 3 mJ is available. Based on a SR-surface of 2 m^2/g, a maximal inside negative membrane potential of -87 mV and a specific membrane capacitance of 1 $\mu F/cm^2$ only about 9 nmol of Ca^{++} can be released without charge compensation before the equilibrium potential is reached. This means that practically the full release has to be compensated by counter current to maintain a substantial driving force during release. The energy derived from the amount of Ca^{++} and its gradient or the caloric value of ATP required for Ca^{++}- uptake (100 nmol) sets an upper limit for specific membrane resistancof the SR in the order of 200 $\Omega\ cm^2$. This is much lower than estimates based on indirect measurements. It is compatible with values obtained from fusing SR with lipid bilayers and the idea that the SR functions as current source rather than as a voltage source.

S5 18

Creatine kinase (CK) and phosphocreatine (CP)-shuttle updated

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The primary source of energy for contraction is ATP hydrolysed by the actin-activated Mg^{2+}ATPase which is regulated by Ca^{2+}. However, eventhough cellular pools of ATP are rather small no significant change in ATP is detected during contraction, for ATP is continuously replenished by the CP-CK system. CK itself is functionally and compartmentally coupled to glycolysis and oxidative phosphorylation, the two ultimate ATP generating pathways. Evidence has been accumulated that the so called "soluble" CK isoforms are in part localized specifically at places of high ATP demand (myofs., SR, etc.) and high ATP production (mitochondria) the CK isoforms communicating by a intracellular CP-shuttle. An account on the present state of the CP-shuttle model is given including recent results by ^{31}P-NMR and analysis of sperm motility which turned out to be an exciting model to test the validity of the theory. In addition, some recent results on the octameric nature of mitochondrial $(MiMi)_4$-CK that fit well into the energy channeling function of Mi-CK are presented.

S5 19

REGULATORY LIGHT CHAIN FUNCTION IN SKELETAL MUSCLE MYOSIN

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The regulatory light chain (RLC) of myosin is able to bind one Ca^{2+} and thus regulates contraction in molluscan striated muscles. In vertebrate smooth muscle and in non-muscle cells the RLC regulates contraction by reversible phosphorylation. In vertebrate striated muscles, including heart, regulation is effected by Ca-binding to the troponin system on the actin filaments. Here, the RLC seems to play a modulatory role only. We have measured Ca- and Mg-binding to RLC in intact myosin. In the absence of divalent metal ions the RLC can be removed by limited proteolysis. Without both RLC the two globular head portions of myosin aggregate affecting its hydrodynamic behaviour. This interaction is non-covalent, hydrophobic in nature and not readily reversible. It can only be reversed by reconstitution with intact RLC. Currently we are localizing the region responsible for this type of interaction within the myosin heavy chains.

S5 20

ACTIN: CORRELATION BETWEEN STRUCTURE, FUNCTION AND INTERACTION

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To reveal the 3-D molecular structure of actin filaments (F-actin) computationally straightened stretches from electron micrographs of negatively stained samples have been helically reconstructed. The resulting 3D maps yield reproducible structural detail about the overall size, shape and orientation of the actin subunits within the filament, and therefore allow building of synthetic filaments from higher resolution molecular actin models determined from actin crystals. Furthermore, these maps reveal two major types of intersubunit contacts: one along and the other between the two long-pitch helices defining the 2-stranded filament. Occasional partial unravelling of short filament stretches into their two long-pitch helical strands indicates that the physically stronger intersubunit contacts are along the two long-pitch helices. These structural results are evaluated in terms of polymerization data which provide independent information about the actin-actin interactions as they occur in the filament, as well as between filaments in F-actin paracrystals and bundles. To understand the structural basis of actin's specific interactions with contractile, regulatory and cytoskeletal proteins, mapping of the binding sites for some of these proteins on the actin molecule has been attempted via 3-D structural analysis of crystalline arrays induced from specific complexes between actin and actin-interacting proteins.

S6 21

THE MOLECULAR BASIS OF AXONAL TRANSPORT IN THE SQUID GIANT AXON

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A new motility protein, kinesin, first isolated from squid giant axons is found in many cells. It is able to use ATP to transport latex beads towards the plus ends of microtubules, which would correspond to the direction (anterograde) away from the cell nucleus in neurons. A second translocator, now known to be a dynein, translocates beads in the opposite direction. Video microscopy also reveals new details concerning how kinesin produces movement and shows that it has properties which differentiate it from myosin and dynein. Kinesin also appears to be an organelle motor, but integral membrane as well as extrinsic proteins on the organelle surface are also necessary for organelle movements.

S6 22

SALTATORY PARTICLE MOVEMENTS IN DICTYOSTELIUM DISCOIDEUM

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Saltatory movements in the cytoplasm of D. discoideum amebae possibly play a role in endo- and exocytosis and in the traffic of vesicles involved in other motile activities. Simple forward and complex to-and-fro movements occur. Velocity is size-dependent: small particles (<1µm) move significantly faster than large (~1µm) and very large (>1µm) ones. Small and large particles appear ultrastructurally as vesicles of various sizes; very large inclusions are food vacuoles. Saltatory movements depend on an intact network of microtubules (MTs): they are frequent in the vicinity of the microtubule-organizing center and radial towards or away from it. There is also a good match between track patterns of particles and the MT network revealed by indirect immunofluorescence. Furthermore, saltations do not occur in mitotic amebae that lack the complex of cytoplasmic MTs, but they resume when the interphase MT complex is rebuilt towards the end of cell division. The MT inhibitor nocodazole destroys the delicate MT network, but its effects on the saltatory movements suggest that it does not directly affect the motility motor.

S6 23

DIRECTIONAL MOVEMENT OF PHAGOSOMES IN THE RETINAL PIGMENT EPITHELIUM

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The vertebrate RPE phagocytizes tips of the light-sensitive outer segments that are rhythmically shed from the photoreceptor cells. In the frog *Rana ridibunda*, phagocytosis of rod tips occurs after dark to light transition. Ingested membrane material moves rapidly from the apical to the basal side of the RPE cells. The underlying transport mechanism is likely to be the microtubular system of the RPE cells, since experimentally induced alterations of the microtubular organization inhibits directional movement of the phagosomes. Using antibodies directed against proteins of rod outer segments, we have followed movement and degradation of the phagolysosomes throught the light cycle. By 12 hrs after onset of light, proteolysis of most of the phagosomes is achieved. Supported by SNSF grant N° 3.883.0.85.

S6 24

THE CHROMATOID BODY AS A CARRIER PARTICLE FOR mRNA IN GERM - AND SOMATIC CELLS .

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The chromatoid body (CB) is a motile particle-like organelle up to 0.9 µm in size that was first described in rat spermatocytes and spermatids, and later in the same vertebrate and invertebrate germ cells. CBs were also found in planarian regenerative cells and in human tumour cells of the germ line and somatic origin. The motility of the CB has been most readily studied in the early spermatid of the rat where this organelle produces typical movements which occur around the condensing nucleus, making transient contacts with it. Several investigations including studies of actinomycin D influence have demonstrated the presence of RNA within the CB. In addition actin filaments were found, forming a network which acts as a carrier for long lived RNA. In spermatids, during condensation of the nucleus, RNA synthesis is interrupted but proteins are still being formed. Thus, because of the CB, some proteins like the calyx forming protein (calycin, M_r 60K) for example, could well be synthetized at a late stage of spermatogenesis. It has to be elucidated, if this particle-like organelle acts in a similar way in the diploid regenerative planarian cells and in human tumour cells, where they may contain mRNAs for normal- and for oncoproteins.

S6 25

INTERACTION OF THE GOLGI APPARATUS WITH MICROTUBULES

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Disruption of interphase microtubules (MT) by the MT-depolymerizing drug nocodazole leads to a complete spatial disorganization of the Golgi apparatus (GA) within one hour, with Golgi elements scattered throughout the cytoplasm. The GA reassembles within 30-60 min upon removal of the drug and MT repolymerization. This dynamic process was visualized in living fibroblast cells (Vero) by video-enhanced fluorescence microscopy using NBD-ceramide (Lipsky and Pagano, Science 228: 745, 1985) as a specific marker for the GA. During reassembly, Golgi elements are translocated retrogradely along MT towards the microtubule organizing center (MTOC) with maximal velocities of 0.3 µm/sec. Reeassembly and positioning of the GA in the region of the MTOC is independent of intermediate filaments and microfilaments. A 110KD MT-binding protein which is associated with the GA (Allan and Kreis, J. Cell Biol. 103: 2229, 1986) may play a role in the positioning of the GA in the region of the MTOC. We are testing an *in vitro* system, consisting of MT with defined polarities, a cellular fraction enriched for GA, and soluble, cytosolic factors or purified 110K protein, to analyze the interaction of the GA with MT.

S7 26

ELECTRICAL AND MAGNETIC STIMULATION OF THE HUMAN BRAIN

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The effects of electrical and magnetic stimulation of the brain usually have been described in terms of positive phenomena: i.e. muscle twitches or visual phosphenes from stimulation of motor or visual areas. However, such stimuli also might be capable of disrupting, rather than mimicking normal brain function. We have seen two examples of this type of phenomenon. First, if a just-suprathreshold electrical shock is given to the motor cortex, the e.m.g. response to a magnetic shock given 5-20 ms later can be inhibited. Responses to mono-synaptic spinal (H-reflex) inputs, given at similar intervals after the electric conditioning shock are not inhibited. Thus, the reduction may reflect inhibition at supraspinal levels. Second, if subjects are trained to flex their wrist as rapidly as possible after hearing an auditory tone, interpolation of a suprathreshold electrical or magnetic shock in the reaction interval between tone and response, can delay the voluntary reaction without changing its form, by up to 100 ms. This cannot be due entirely to inhibition or refractoriness of spinal motoneurones since H-reflexes are not suppressed after the cortical shock.

S7 27

ORGANIZATIONAL PRINCIPLES OF PRIMARY AND SECONDARY MOTOR CORTICAL FIELDS OF THE PRIMATE'S BRAIN

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Repetitive electrical stimulation of the brain surface led to the first brain maps, and the method was essential in the definition of the motor cortex. Within the motor cortical fields so defined, a number of specialized subareas were delineated more recently with modern anatomical tracing, microelectrophysiological and behavioural techniques. Current evidence suggests parallel as well as hierarchic processing in primary (area 4) and secondary motor areas (subdivisions of area 6). Comparative results obtained in the various fields with microstimulation and single unit recordings in the performing animal will be presented to exemplify similarities and differences in the organization of the precentral and the supplementary motor cortex.

S7 28

THE MONOSYNAPTIC REFLEX IN REACTION TIME TASKS

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In a reaction time situation, the monosynaptic spinal reflex (H reflex) is facilitated before the onset of an electromyographic response. Even in a choice reaction time situation, the facilitation was specifically ipsilateral to the movement implying that the decision which limb will move was made before the onset of the facilitation. There was no facilitation of motoneurons before movement onset and it was concluded that the basis of the facilitation is an enhanced excitatory effect of the afferent volley elicited by the H reflex stimulus. Mechanisms could be removal of presynaptic inhibition at Ia terminals or facilitation of interneurons of polysynaptic components of the reflex. The duration of the facilitation did not depend on the modality of the go stimulus and the complexity of the task. In contrast to the movement duration, the duration of the facilitation depended, however, on reaction time. This result indicates that the command for the movement and the facilitation are not of the same central origin.

S7 29

MOTOR AND FUSIMOTOR SET IN VOLUNTARY MOVEMENT

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Voluntary movements are generated by hierarchically organized CNS structures, where lower level circuits are controlled from higher order centres. For instance, isolated spinal networks are capable of producing the backbone of rhythmic motor output, but during normal movement their performance is modulated via descending pathways from brain stem and motor cortex. Whilst this general notion is widely accepted the question of the nature of the descending signals (motor commands) is entirely open. In the past the emphasis has been on rhythmic neuronal discharge, as recorded from various sensorimotor centres during rhythmic movement. However, Evarts et al. (Neurophysiol. Approaches to Higher Brain Function, N.Y.: Wiley, 1984) have recently drawn attention to the importance of tonic gating signals for higher order control of lower level motor structures. Their notion of motor set implies that in part motor output is shaped by setting and resetting of gains in networks featuring parallel pathway redundancy. Set operation is also observed in the peripheral motor system. The concept of fusimotor set describes control of muscle spindle feedback in terms of essentially tonic, but adjustable, levels of γ-activity, with low-key γ_S action prevailing in routine tasks, and strong γ_D action occurring in unfamiliar movements.

S8 30

GAP JUNCTIONS AND INTERCELLULAR COUPLING : A MORPHOLOGICAL APPROACH

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Gap junctions mediate direct communications (coupling) between adjacent cells. By electron microscopy, they are sites where closely apposed membranes contain aggregates of uniform and randomly distributed (by optical diffraction) 9 nm particles. In light microscopy, they are immunostained with antibodies against 21 and 26 kD peptides. Junctional coupling can be visualized by introducing into cells membrane-impermeant molecules whose intercellular transfer can be monitored by autoradiography or fluorescence microscopy. Using these techniques, we have quantitated gap junctions and coupling in the endocrine and exocrine pancreas. These studies have revealed a close relationship between the modulation of junctional coupling and the control of insulin and amylase secretion, thus providing evidence for a role of gap junctions in the physiological function of two highly differentiated gland cell systems.

S8 31

BIOCHEMICAL ASPECTS OF INTERCELLULAR COUPLING

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Gap junctions are sieve-like structures which join the cytoplasms of adjacent cells in most tissues of metazoan animals. Their structure and permeability have been highly conserved during evolution. They were first isolated twenty years ago by Benedetti and Emmelot but, surprisingly, there is still uncertainty about the identity of the component proteins. To review the current situation, junctions may be made from a tissue invariant protein of molecular weight 16,000 to 18,000, which is highly conserved across species and phyla (including arthropoda). Alternatively vertebrate junctions from different tissues may contain different proteins of a divergent family, varying in molecular weight from 25,000 to 45,000 with no known arthropod counterpart. Antibodies raised against isolated junctions and specific proteins from both classes bind to morphologically identifiable gap junctions and block junctional communication when injected into sensitive cells. Careful use of immuno- and chemical inhibitors is however providing new information on the functional importance of junctional communication in animal tissues.

S8 32

ELECTRICAL PROPERTIES OF CELL-TO-CELL CHANNELS IN COUPLED PANCREATIC ACINAR CELL PAIRS

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The properties of the junctional conductance of electrically coupled cell pairs were studied by using the double whole cell patch-clamp technique. Acinar cell pairs were isolated from the mouse pancreas by collagenase treatment. The initial electrical coupling corresponds to the simultaneous opening of 400-600 cell-to-cell channels of 130 pS. After about 15-20 min, the cells uncouple spontaneously and conductance steps of about 130pS become occasionally visible. The spontaneous uncoupling can be suppressed by use of cAMP (0.1 mM) and ATP(5mM) containing pipette filling solutions. Uncoupling can be achieved by addition of protein kinase C to this pipette filling. At conditions of stable cell coupling addition of 80 nM OAG to the bath induces rapid electrical uncoupling. The data provide evidence that protein kinase A and C dependent cytoplasmic reactions are responsible for cell-to-cell coupling and uncoupling, respectively.

S8 33

ELECTRICAL PROPERTIES AND ANION PERMEABILITY OF DOUBLY RECTIFYING JUNCTIONS IN THE LEECH CNS

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Electrical connections between touch sensory neurons in the leech CNS are known to be double rectifying: depolarization spreads equally well in both directions whereas hyperpolarization spreads only poorly in either direction. To test whether this is due to the junction itself or whether particular current passing properties of extrajunctional membrane is involved, one T cell was stimulated repetitively in order to create a pump induced hyperpolarization in the cell body and its processes in the neuropile (junctional site): Hyperpolarization still failed to spread to the coupled T cell whereas depolarization still did. Further evidence for double rectification of junctions linking T cells was provided by using reversing IPSP'S as an assay for increased intracellular Cl concentration: Cl (injected into one T cell) was found to move through the junction and cause the IPSP'S in the coupled T cell to reverse. Cl movement through T-T junctions was also found to show the same voltage dependence as current flux: Cl failed to move when either Pre-, or postsynaptic cell were held hyperpolarized at -95 mV whereas it immediately started to move when the membrane potentials were brought back to their original value at -45 mV.

S8 34

IMPULSE PROPAGATION AND ELECTRICAL COUPLING IN CARDIAC TISSUE

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In cardiac tissue impulse propagation requires local circuit currents which are determined by excitability parameters and passive electrical properties. Experimental work will be presented which describes the passive electrical properties of intact cardiac tissue and isolated myocyte pairs. The former involves microelectrodes and conventional cable analysis, the latter patch pipettes in the whole-cell, tight-seal recording mode. Utilizing cell pairs, current-clamp experiments enabled the study of the transfer of action potentials from cell to cell, while voltage-clamp experiments assayed the electrical properties of the nexal membrane. Combining both approaches it was possible to correlate the functional state of impulse transfer with the nexal membrane resistance, r_n. Complementary experiments investigated the electrical properties of cell-to-cell channels. For this purpose cell pairs were exposed to heptanol (1-3 mM), octanol (1 mM), or halothane (4 mM) to reduce reversibly the number of operational connexons. Under this condition, the single channel conductance was found to be 40-80 pS. The current-voltage relationship of the channels was linear (range: -100 to +100 mV)

S9 35

IDENTIFICATION OF A DNA SEQUENCE REQUIRED FOR A HIGH FREQUENCY OF MEIOTIC GENE CONVERSION AT THE ARG4 LOCUS OF *S. cerevisiae*.

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Previous genetic studies at the ARG4 locus in *S. cerevisiae* (Fogel *et al.* CSHSQB 43:1325-1341, 1979) have shown that the frequencies at which different mutant sites in the gene undergo meiotic gene conversion diminish from one end of the gene to the other. It has been hypothesized that this initiation site near the high conversion end of the gene. We have re-examined this phenomenon using defined mutations within the ARG4 gene as an initial step towards identifying DNA sequences that may be responsible for high frequencies of gene conversion. Seven arg4 $^-$ alleles constructed *in vitro* were examined by tetrad analysis in mutant by wild type crosses. Within 1271 nucleotides, gene conversion frequencies ranged from 9.6% at the 5' end of the gene to 0.6% at the 3' end. Two mutant alleles, RV at the high conversion end and Bgl at the low end, were used to assay the effect of ten homozygous deletions that span a 10 kbp region surrounding the ARG4 gene. These studies define a region near the 5' end of the gene that appears to be responsible for high frequencies of gene conversion. This 320 bp region includes the ARG4 promoter. Further characterization of this gene conversion element and its properties will be presented.

S9 36

ANALYSIS OF RECOMBINATION INITIATION IN FISSION YEAST

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The genetic recombination during meiosis is not evenly distributed over a chromosome. Experimental evidence suggests specific DNA sequences at which recombination are initiated. The mutation ade6-M26 in a gene of the purine pathway of S. pombe shows peculiarities in recombination assays. It was postulated that M26 creates a preferred initiation site for recombination. We sequenced the ade6 wild-type gene, the M26 and other mutant alleles. The M26 mutation is a single basepair substitution which presumably improves the recognition by the recombination enzymes. So far, it was only shown that M26 stimulates nonreciprocal recombination (Conversion). With an artificial heteroalleleic duplication we could show that M26 also increases the frequencies of crossing-overs. Following current models for recombination mechanisms, it was suspected that near M26 site-specific DNA cuts could occur. We tested this by anlyzing DNA of cells undergoing synchronous meiosis. But up to now, no such cuts could be detected. In order to establish the extent and nature of the responsible sequence around M26, we started to generate base substitutionsby in vitro techniques. The results indicate that a specific sequence around M26 is responsible for the increase of recombination frequency.

S9 37

REC-DNA COMPLEXES AND STRAND EXCHANGE

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The link between the polar structure of recA-DNA complexes and the polar action of recA protein during recombination has been established by demonstrating that recA binds to single-stranded DNA with an unique polarity. This result together with direct visualization of recombining molecules completes a molecular model of the recA-mediated process of general genetic recombination.

S9 38

HOMOLOGOUS RECOMBINATION AND GENE TARGETING IN PLANT

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Nicotiana tabacum SR1 protoplasts were cotransformed by the method of the direct gene transfer with pairs of plasmids, carrying deletions of the selectable marker, neomycin phopho-transferase (NPT II), gene. The full size functional marker gene could be restored by homologous recombination within the provided region of homology (0-400 bp). The pretreatement as well as the physiological structure of the incoming plasmids have a influence on the recombination frequency. Supercoiled (circular) plasmids recombine with at least a 10 fold lower frequency then linear ones. Linearisation of one increased the frequency only slightly. If both plasmids were linearised, the recombination frequency was highest and increased proportionally with the length of the homologous region provided for recombination. Linearisation of the molecules near the homologous region gave the highest frequencies. The recombination of foreign DNA into a homologous region on the chromosome (gene targeting) was successful but occured with a relatively low frequency (1 per 10000 transformants).

S9 39

DNA MISMATCH REPAIR IN E. COLI AND EUCARYOTES.

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Base-base mismatches arise from replication errors, as well as from recombination and deamination events. In E. coli, mismatch correction is biased mainly towards the repair of replication- and deamination-associated mispairs. In the former pathway, the repair is directed to the newly-synthesized strand, which is transiently under-methylated on adenine residues within the tetranucleotide GATC. The latter type of lesion, a G/T mispair, arises as a result of 5-methylcytosine deamination, and is invariably corrected to a G/C, irrespective of the adenine methylation status. Mispairs arising from recombination events appear to be largely unaddressed in this system.
The investigation of mismatch repair efficiencies in a simian system revealed that the G/T mispair is efficiently corrected to restore a G/C pair. Other mispairs were found to be corrected with low efficiency. These results suggest that mismatch correction in primates acts primarily to maintain the pattern of cytosine methylation.

S10 40

ORGANIZATION AND MODIFICATION OF GENES FOR PROTEINS OF THE PHOTOSYNTHETIC APPARATUS.

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The photosynthetic membrane is composed of ca. 50 polypeptide species, most of which are found in four multisubunit complexes. Approximately 40 % of them are encoded in plastid chromosomes, the remainder originates in the nucleus. The functional organization of plastome-encoded genes is complex. The genes are organized in nine operons. Multiple-banded RNA patterns that include long polycistronic transcripts arise by post-transcriptional modification. At least four discrete mechanisms for RNA modification can be distinguished. The biogenesis of membrane complexes is regulated at various stages. Light can operate at different levels in plastids and nucleus/cytosol, respectively. The components of nuclear origin are decoded on cytosolic ribosomes as precursors. Their transit sequences exhibit little similarity in primary structure but some of them share discrete secondary structures that may be involved in intracellular sorting and intraorganelle routing. The development of a cassette system that simplifies exchange and modification of gene domains, and the use of that system to study reciprocal exchanges of transit sequences and to explore structure/function relationships in photosynthesis by compartment-alien transformation of appropriate Oenothera mutants or anti-sense RNA will be discussed.

S10 41

THE TOPOLOGY OF THE MEMBRANE PROTEINS OF PHOTOSYSTEM II

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The protein core of photosystem II consists of six integral polypeptides that contain the binding sites for the reaction center chlorophyll P680, antenna chlorophylls, two pheophytins, two plastoquinones, two cytochromes b-559, Fe and Mn. The D-1 and D-2 subunits carry the reaction center and the redox components for electron flow from Mn to plastoquinone Q_B, the 9 and 4 kDa subunits cytochrome b-559 and the 44 and 47 kDa subunits the core antenna chlorophyll. The primary amino acid sequence of all polypeptides is known. The folding of the polypeptide chain is predicted from hydropathy analysis. The homology of the reaction center polypeptides D-1 and D-2 to the equivalent polypeptides L and M respectively in the reaction center of purple bacteria and of the hydropathy index plots reveal the conservation of essential amino acids in the binding of the redox components, like his 198 for the reaction center chlorophyll or his 215 for Fe binding. DNA-sequencing of mutants of herbicide tolerant plants yielded amino acids involved in plastoquinone binding and site directed mutagenesis identified tyrosine 160 involved in the primary electron donor for the reaction center. Detailed models will be presented.

S10 42

Molecular genetics of chloroplast-nucleoplasmic interactions in Chlamydomonas reinhardtii.

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The biosynthesis of the photosynthetic apparatus of higher plants and algae depends on a close cooperation between the chloroplast and nucleocytoplasmic genetic systems. In order to study this process we have isolated and characterized several nuclear and chloroplast mutants of Chlamydomonas reinhardtii deficient in photosystem II (PSII) and photosystem I (PSI) activity. Analysis of the transcript and protein levels and of the rate of protein synthesis in these mutants has provided new insights into the assembly of these photosynthetic complexes. Surprisingly the expression of some chloroplast genes is specifically and individually controlled by several nuclear factors either at the level of RNA maturation or translation/post-translation. One possible way to identify these regulatory factors is to clone their genes by transposon tagging. We have recently isolated and characterized a movable element from C. reinhardtii which may be useful for this purpose.

S10 43

ORGANIZATION OF GENES INVOLVED IN CHLOROPLAST PROTEIN SYNTHESIS : A MINIREVIEW

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According to extensive DNA sequencing data the chloroplast genomes of lower and higher plants carry genetic information for a complete set of rRNAs, a minimal but sufficient (function) number of tRNAs, for about one third of ribosomal proteins and a few protein factors required in translation. A comparison of these data reveals that number and kind of genes concerned are about the same both in green algae and higher plants, while the organization and arrangement of these genes underwent considerable changes in many cases. As a consequence size and composition of transcription units for homologous genes are distinct offering the possibility to study expression of homologous genes (transcriptional regulation) in different genomic environments.

(Collaborators : J.-M. von Allmen and P.E. Montandon)

S11 44

PROTEIN STRUCTURE DETERMINATION BY X-RAY DIFFRACTION OF SINGLE CRYSTALS

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X-ray crystallography was established as a technique to determine accurate spatial structures of proteins in the late 1950's by M.F. Perutz and J.C. Kendrew. Since 1959, when the first protein structure (myoglobin) was solved, data collection techniques and computing facilities have dramatically improved. This resulted in more and more frequent publications of structures of proteins of ever increasing size and complexity, culminating in large assemblies like viruses and the nucleosome. The amount of time required to solve a protein structure has also decreased considerably in recent years. The newest technological improvements allow the successful elucidation of a protein structure in less than half a year in favourable cases.

The interest in X-ray crystallography has intensified when it was realised that in order to understand the function of the products of all the newly cloned genes knowledge of their spatial structure is required. The number of protein crystallography groups is correspondingly increasing, both at universities and in industry.

Some recent advances in methodology will be discussed and illustrated with projects carried out at the Biozentrum.

S11 45

NUCLEOSOME CORE PARTICLE: X-RAY CRYSTALLOGRAPHIC STUDIES ON A MULTI-COMPONENT PROTEIN-DNA COMPLEX

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The nucleosome core particle contains 146bp of DNA wrapped around four pairs of histone proteins. It is the greater part of the nucleosome, the fundamental repeating element of eucaryotic chromatin. This 206,000Da nucleoprotein-DNA complex was crystallized after release from whole chromatin by nuclease digestion, and the structure was determined at 7Å resolution by X-ray crystallography (Richmond, Finch, Rushton, Rhodes, & Klug, *Nature* **311**, 532). To improve the resolution of the structure, the histone octamer protein complex has been reconstituted with a recombinant segment of the 5S RNA gene from *Lytechinus variegatus*. Crystals obtained from this material containing defined sequence DNA show diffraction limits at 3.0 - 4.5Å as compared to those of 3.5 - 6.5Å for the original crystals with mixed sequence DNA. The crystallographic solution of the nucleosome core particle, and now other large protein complexes, has been possible using multiple isomorphous replacement in conjunction with compounds containing several heavy atoms.

S11 46

REFINEMENT OF THE STRUCTURE OF TENDAMISTAT DETERMINED BY NMR IN SOLUTION AND COMPARISON WITH THE CRYSTAL STRUCTURE

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The protein Tendamistat (MW ≈9000) inhibits mammalian α-amylases by tight binding. Its three-dimensional structure was recently determined independently by nuclear magnetic resonance in solution (Kline et al., J.Mol.Biol. 183, 503 (1985); 189, 377 (1986)) and by X-ray diffraction in single crystals (Pflugrath et al., J.Mol.Biol. 189, 383 (1986)). Comparisons of the solution conformation with the crystal structure were done using the structures obtained directly as the output of the distance geometry calculations with the program DISMAN, as well as with structures obtained after restrained energy refinement of the DISMAN output using a modified version of the program AMBER. Overall the molecular architecture was found to be closely similar in single crystals and in solution; a description of local differences is the focal point of this presentation.

S11 47

THEORETICAL APPROACHES TO PROTEIN DESIGN

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S12 48

CYTOCHROME OXIDASE AS A REDOX-DRIVEN PROTON PUMP

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Cytochrome oxidase (EC 1.9.3.1) is the terminal member of the respiratory chain in mitochondria of eukaryotes, and in some aerobic bacteria. It catalyses the oxidation of cytochrome c by O_2, and is thus the ultimate "respiratory enzyme" which is responsible for more than 90% of the biological oxygen consumption on Earth.

Cytochrome oxidase also catalyses generation of protonmotive force across the inner mitochondrial (or bacterial) membrane, a process that is linked to and driven by the electron transfer to dioxygen.

Generation of protonmotive force by the enzyme can be delineated as two separate charge-translocating processes coupled in series. One of these, the so-called proton pump, links electron transfer between cytochrome c and the enzyme's binuclear haem iron-copper centre to translocation of H^+. The other results from the property of the binuclear centre to accept electrons from one side and protons from the other side of the membrane in the catalysis of O_2 reduction to water. The latter process has recently been elucidated in some detail, and will be discussed.

S12 49

THE FRONT EDGE SEEN FROM THE REAR END. A HISTORICAL FOOTNOTE TO THE CALCIUM PUMP

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S12 50

ASYMMETRIC DISTRIBUTION OF Na^+ TRANSPORTERS IN EPITHELIAL CELLS

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Na,K-ATPase cDNAs for α and ß subunit from Xenopus laevis kidney epithelial cells (A6) were obtained by screening expression libraries with polyclonal antibodies directed against both enzyme subunits. A third cDNA coding for a distinct membrane protein was identified by the anti-α subunit antibody. The three fusion proteins were used to raise polyclonal antibodies. Immunoprecipitation from pulse labeled A6 cells demonstrated that association of Na,K-ATPase subunits takes place early during enzyme biosynthesis. Immunoprecipitation from A6 cells, selectively labeled at the apical or the basolateral surface, demonstrated that Na,K-ATPase was restricted to the basolateral membrane of the cells, while the third anti-fusion protein antibody immunoprecipitated proteins labeled at the apical surface. The nucleotide sequence from near full length cDNAs for both enzyme subunits was determined. Sequence comparisons show that the α subunit is an α (αI) isoform and that both subunits have a higher degree of similarity to mammalian than fish sequences.

S12 51

MUTATIONAL ANALYSIS OF THE YEAST H^+-ATPase

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A test system for in vitro generated mutants of the yeast plasma membrane H^+-ATPase has been deviced. The wild type chromosomal gene is under a galactose-dependent promoter while the mutagenized ATPase has a constitutive promoter. As the ATPase is essential for yeast growth, growth on glucose medium depends on the activity of the mutagenized enzyme. Directed mutagenesis of 14 amino acids conserved in all the ATPases with phosphorylated intermediate has dissected the enzyme into kinase, transduction and phosphatase domains. Surprisingly, the aspartate residue forming the phosphorylated intermediate is not essential. Random mutagenesis with hydroxylamine resulted in temperature-sensitive mutants which mapped in the regions conserved in all the ATPases with phosphorylated intermediate. One of the temperature-sensitive mutants, mapping in the kinase domain, was also deffective in the physiological activation of the ATPase by glucose. This work illustrates the advantages of yeast as model system in the Molecular Biology of Eukaryotic cells.

S12 52

STRUCTURE OF THE K^+-ATPase OF STREPTOCOCCUS FAECALIS

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We cloned the gene of the K^+-ATPase from S. faecalis on a pUC8 vector in E. coli, using antibodies against the ATPase. The gene encodes a protein of 583 amino acids and a calculated molecular weight of 63,070 Da. It exhibits 25% homology to the KdpB-subunit of the K^+-ATPase of E. coli and, to a lesser extent, to eukaryotic ion-motive ATPases. The S. faecalis enzyme also contains the sequence asp-lys-thr-gly-thr that has been found in all other ATPases for which sequence information is available. This pentapeptide contains the aspartic acid residue that becomes phosphorylated as part of the reaction mechanism and appears to represent a universal feature of the active site of ion-motive ATPases. The hydrophobicity profile of the S. faecalis ATPase suggests the presence of six transmembraneous helices, with the bulk of the protein facing the cytoplasm. Future work will be aimed at elucidating structure-function relationships on this model system for ATP-driven ion translocation.

S12 53

REGULATION OF THE Ca-ATPase OF SARCOPLASMIC RETICULUM (SR)

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The major protein constituent of the SR membrane is a Ca-dependent ATPase, whose activity is coupled with the vectorial translocation of Ca from the cytosol into the intracellular compartment. This transport activity is responsible for the relaxation of striated muscles. Activation of Ca-release channels located on the SR membrane allows Ca-ions to flow back to the cytosol, thus inducing contraction. cAMP (a well known modulator of myocardial activity) and calmodulin stimulate in an additive way (up to 5-fold) the rate of accumulation of Ca by cardiac SR membranes. Both modes of action involve indirect mechanisms leading to the phosphorylation of phospholamban (PLB), an intrinsic regulatory protein of the SR membrane. High resolution SDS-gelelectrophoresis combined with isoelectrofocusing analysis have yielded information on the properties of PLB. The PLB complex (Mr of about 25 kDa) is composed of 5 probably identical subunits, each containing a distinct phosphorylation site for the calmodulin- and the cAMP-dependent kinase. However, two functionally distinct populations of PLB exist, which respond differently to the action of the two kinases. The two populations have a specific distribution within the SR network, such that the Ca-pump of the cisternal compartments is sensitive to calmodulin while the longitudinal system is regulated by cAMP.

S13 54

GENETICALLY ENGINEERED VACCINES : RECENT DEVELOPMENTS.

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Vaccines have rapidly become prefered targets of genetic engineering and a huge effort has been made in the last ten years but it clearly became obvious that the development of such vaccines would be long and complex. Until now, with the exception of veterinary vaccines against enterotoxins, the human vaccine against hepatitis B is the only one which can be considered as a success. Nevertheless, the use of DNA recombinant technology remains the best approach to elaborate new vaccines against numerous viruses (AIDS, ...) and parasites (malaria, ...) and we can expect attractive results in the near future, especially when the need is absolute. Results obtained by Transgene in the case of rabies are, for instance, very promising and spectacular ; the new vaccine is based on a recombinant vaccinia virus expressing the rabies glycoprotein. The vaccinia virus is of course an attractive carrier system for other antigens (HIV, ...). Genetic engineering is in fact a powerful method to dissect the immunological response of an animal invased by various foreign organisms. Parasites are certainly the best illustration and recent results in this field will be presented and discussed.

S13 55

WHAT CAN CLONED AND EXPRESSED ANTIGENS FROM *ECHINOCOCCUS MULTILOCULARIS* METACESTODES BE USED FOR?

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The metacestode of *Echinococcus multilocularis* is the causative agent of alveolar echinococcosis in man and rodents. Due to the tumour-like proliferation of the parasite, this severe disease requires early diagnosis for effective surgical treatment; moreover host-parasite interactions need to be investigated with regard to immunological functions of parasite antigens. Such works require large amounts of purified parasite antigens. A promising approach in this direction can be done by expressing cloned gene fragments from *E. multilocularis*. Using suitable cloning and expression vector systems the gene products (parasite antigen polypeptides) can be used for immunodiagnostic purposes and for investigating humoral and cellular immune response of infected intermediate and definitive hosts. Another potential value is discussed which consits in selecting protective antigens and expressing the adequate genes in a way to induce a host B- and T-lymphocyte sensibilization (e.g. introducing gene fragments by plasmids into life attenuated *Salmonellae*).

S13 56

The surface protease of *Leishmania* promastigotes.

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Promastigotes of Old and New World *Leishmania* express an abundant surface membrane glycoprotein of 59 to 72 kDa that was first identified by surface radioiodination, immunological crossreactivity, peptide mapping, and more recently by its neutral-to-alkaline protease activity. The evolutionarily conserved proteolytic enzyme, designated as the promastigote surface protease, or PSP, can be labeled *in vivo* with [^{3}H]-myristic acid or [^{3}H]-palmitic acid. A soluble form of the protease can be generated *in vitro* by the action of PI-specific phospholipase C with the concommitant loss of the radioactive fatty acid label and the exposure of the epitope recognized by antibodies to the cross-reacting determinant (CRD) of the soluble form of the variant surface glycoprotein (VSG) of *Trypanosoma brucei*. The lipase sensitivity and the presence of the CRD on the soluble form of PSP shows that it is anchored to the membrane by a glycophospholipid antigenically indistinguishable from the membrane anchor of the major surface protein of the salivarian trypanosomes.
Although evidence indicates that the promastigote surface protease occurs at the surface of the parasite in the midgut of the phlebotomine sandfly vector, the activity of the enzyme may also be essential during the process of macrophage invasion in the mammalian host.

S13 57

A MICROTUBULE-ASSOCIATED-PROTEIN IN THE MEMBRANE SKELETON OF TRYPANOSOMA BRUCEI

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The membrane skeleton of the parasitic protozoon Trypanosoma brucei consists of a dense array of microtubules, which are vertically connected to the cell membrane and laterally to each other. We here describe a protein (p270) from Trypanosoma brucei, which behaves as a microtubule (MT)associated protein (MAP): It binds in vitro to preformed MT and it is colocalized with the subpellicular MT. This was shown by double-immunogoldlabelling done on negative stained cytoskeletons with anti-tubulin and anti-p270 antibodies. p270 shares the following properties with brain MAP-2: (i) it shows a similar molecular weight, (ii) it is heat resistant, (iii) it shows reciprocal crossreactivity with brain MAP-2. (iv) rod shaped structure in rotary shadowed micrographs. By heat treatment, hydrophobic chromatography and gel filtration it was possible to purify the 270 kd protein. In addition using the Lambda gt-11 expression system we could isolate and characterize sequences coding for the gene of p270.

S13 58

GENETIC RECOMBINATION IN AFRICAN TRYPANOSOMES

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The question of whether African trypanosomes undergo sexual processes is of major interest, especially with respect to the evolution and epidemiology of this parasite group, which is the causative agent of sleeping sickness in man and Nagana disease in cattle. Sexual processes involving gene reassortment are well known among sporozoan parasites such as Plasmodia and Coccidia, but probably the most important factors in eucariotic evolution -meiotic recombination and gene assortment linked to sexual conjugation- have not yet been found in African trypanosomes. Still, there is now evidence for the existence of sexual processes occurring in the parasite's insect vector. This was demonstrated under laboratory conditions for the first time by a group from the Swiss Tropical Institute in collaboration with other groups. The simultaneous cyclical transmission of two Trypanosoma (T.)brucei clones led to cloned progenies showing, among other parameters, hybrid characteristics with respect to isoenzyme and DNA hybridization patterns.

S14 59

The RepC protein of the broad host range plasmid RSF1010 binds specifically to the ori region and is involved in copy number - and replication control.

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RSF1010, a small 8685 bp non-conjugative plasmid of the incompatibility group IncQ, has the remarkable ability to replicate in a very wide range of Gram-negative bacterial species. Its copy number in E. coli and Pseudomonas putida is 12 - 13 per chromosome equivalent. The plasmid encodes its own essential replication genes repA, repB, and repC, which make it independent of host functions required for the primosome formation such as dnaB, dnaC, dnaG, and rpoB. The rep genes of RSF1010 were cloned onto controlled expression vectors and the replication of a coresident RSF1010 replicon was monitored using DNA:DNA hybridization, during amplification of the individual rep genes. Over-expression of repC strongly increases the copy number of RSF1010. RepC was shown to be involved in the positive regulation of RSF1010 replication. It binds specifically to a small DNA segment of oriV which contains three perfectly conserved 20 bp repeat units. The regulation of expression of the rep genes seems to be crucial for the ability of RSF1010 to replicate in a broad range of bacterial hosts.

S14 60

PROTEIN-DNA INTERACTIONS AT THE ENDS OF TRANSPOSABLE ELEMENT IS1

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The minimum sequences required for IS1 transposition consist of the terminal 23-25 bp of either end of the element. These sequences are recognized by two proteins: IHF, a host encoded histone-like protein and InsA, an IS1-encoded protein essential for transposition. Examination of the transposition and protein-binding behavior af a collection of mutant ends show that the ends of IS1 are composed of two domains: the inner domain contains the determinants for specific binding of the proteins while the outer domain contains other determinants of transposition activity. The specific binding determinants of the two proteins overlap extensively. We will also discuss how simple plasmid and gel electrophoresis tools can be used to investigate the specifity of IHF-DNA interactions and their impact on DNA conformation (protein-induced DNA bending).

S14 61

BIOCHEMICAL AND GENETICAL ANALYSIS OF THE BACTERIOPHAGE P1 SITE SPECIFIC RECOMBINATION SYSTEM' CIN.

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The expression of alternative sets of tail fiber genes in phage P1 is regulated by site specific recombination. This recombination is mediated by the phage coded recombinase Cin. For efficient recombination, a 70bp long recombinational enhancer sequence is required in cis, but its orientation and position with respect to the recombination site is unimportant. Using an in vitro system, we have purified the recombinase Cin and a bacterial host factor termed FIS(factor fot inversion stimulation), which stimulates recombination more than 500 fold. DNase I footprinting studies showed that Cin specifically binds to the recombination site and FIS binds to the recombinational enhancer.
In a genetic screening, independent point mutants of Cin were isolated. Intragenic complementation between some of these mutants was found in vivo.

S14 62

CENTROMERES IN SACCHAROMYCES CEREVISIAE

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We want to study the attachement of chromosomes via their centromere to spindle fibers and have begun in a first step with the analysis of DNAs and proteins involved in centromere assembly in S. cerevisiae. All so far isolated 14 Saccharomyces CEN DNAs have a characteristic sequence of slightly less than 120 bp in common. Two highly conserved DNA elements PuTCACPuTG (CDEI) and TGT.T.TG.TTCCGAA.....AAA (CDEIII), flank a very AT-rich stretch of 75-86 bp (CDEII). This DNA segment of 120 bp is sufficient for the assembly of functional centromeres. Several of the highly conserved base pairs in CDEI and CDEIII were changed by oligonucleotide directed mutagenesis. These mutations affected the assembly into centromeres to various degrees. Protein binding to CEN DNA was directly demonstrated in vitro by footprinting techniques and by gel mobility retardation assays and in vivo by determining sizes of "run against" transcripts originating from a promoter cloned adjacent to CEN DNA and by methylation protection detectable with genomic sequencing techniques.

S14 63

SPECIFIC SITES OF DNA BINDING TO NUCLEAR SCAFFOLD OF YEAST

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The nuclear scaffold, or matrix, is proposed to maintain DNA in a looped conformation in both interphase and metaphase chromosomes. Specific DNA fragments, termed SARs, show a high affinity for binding to the isolated nuclear scaffold. In *Drosophila* these were found 5' and 3' from many highly expressed genes (MIRKOVITCH ET AL., 1984; GASSER AND LAEMMLI, 1986). In yeast we find that autonomously replicating sequences (ARS), which appear to serve as origins of chromosomal replication, are constitutively scaffold-attached. Moreover, the origin of replication and the cis-acting sequence required for mitotic stability of the endogenous 2 micron plasmid are scaffold-bound. Deletion mutagensis of these specific sites of attachment will identify the domains required for scaffold interaction and will determine whether scaffold association is necessary for the region to function as an origin of replication. Several of these yeast sequences show striking sequence homology to *Drosophila* attachment sites. Remarkably, we have found that several *Drosophila* SARs have ARS function in the fission yeast, *S. pombe*.

S14 64

Expression of the S. cerevisiae acid phosphatase gene PHO5 implicates physical interaction of two regulatory proteins the PHO2 and PHO4 with the promoter DNA.

K. Vogel (Biotechnology, Ciba-Geigy, Basel)

PHO5 is a regulated gene in S. cerevisiae which encodes an acid phosphatase. High levels of inorganic phosphate lead to a repression whereas low levels of inorganic phosphate derepress the gene. Mutations in PHO2 or PHO4 are epistatic to all other regulatory mutations and show a negative phenotype on low phosphate media which indicates that PHO2 and PHO4 proteins might act as positive transcription factors. Since an over expression of PHO4 protein in a pho2⁻ yeast strain can complement the pho2 mutation and vice versa it is likely that both proteins act at the same level of regulation. PHO5 promoter deletions showed that besides the TATA-box three other boxes on the promoter are required for a fully active PHO5 promoter. It is obvious that one or both of the two proteins (PHO2, PHO4) could interact with these boxes.
In gel mobility shift assay it could be shown that PHO2 as well as PHO4 bind to DNA. In addition it could be shown by using the various PHO5 promoter deletions that PHO4 binds to two of these boxes whereas PHO2 binds to a box situated between the PHO4 binding sites.

S14 65

INTERPLAY OF DNA SEQUENCE ELEMENTS AND CELL TYPE-SPECIFIC FACTORS IN MAMMALIAN GENE TRANSCRIPTION

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A conserved "octamer" (= decanucleotide) motif ATGCAAATNA binds to an ubiquitous and a lymphoid cell-specific transcription factor (OBF-1 and OBF-2). This motif is an important determinant of lymphoid cell specificity. Curiously, it is also present in generally active genes, e.g. histone H2B. We have linked the H2B octamer to a heterologous TATA box. This construct is lymphoid-specific, but regains general activity when associated with other elements from the H2B promoter. Therefore, it seems that lymphoid specificity is the ground state of the octamer, and a complex interplay with other motifs is required to make it a general element. The immunoglobulin heavy chain enhancer harbors further conserved sequence motifs ("Ephrussi" boxes). One of these motifs may be involved in negative regulation of transcription.

S14 66

FUNCTIONAL ANALYSIS OF A TRANSCRIPTIONAL MODULATOR PROTEIN

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We used the glucocorticoid receptor (GR) and its target enhancer (GRE) as a model to develop improved tools for studying trans-activation. We developed three powerful techniques with this system. a) An extremely sensitive transactivation assay: this will be the basis for a genetic screening of revertant transcriptional activators. b) The construction of a mutagenesis "cassette": to this purpose, we entirely re-designed the c-DNA encoding the essential GR functions, in a way that allows rapid generation and direct in vivo testing of specific mutants. c) The linkage to the GR of an additional DNA binding domain derived from an unrelated protein: this allows the generation of bi-functional trans-activators which can be used to better understand the phenotypes obtained by site-directed mutagenesis, with the help of a cis-trans or a trans-trans complementation assay. Perspectives: since the results indicate that it is possible to generate chimaeric GR molecules with the desired additional specificity, we plan to use this fact for the direct selection of further DNA binding factors from mammalian cells.

S14 67

THE DNA BINDING PROPERTIES OF AN *ANTENNAPEDIA* POLYPEPTIDE EXPRESSED IN *E.COLI*

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The *Antennapedia* complex is a cluster of homeotic genes that are involved in the specification of segment identity in *Drosophila melanogaster*. The *Antennapedia (Antp)* gene in this complex contains a homeobox, i.e. a sequence of approximately 180 bp that is characteristic for many *Drosophila* genes specifying the body plan, and which is also present in a wide variety of other organisms including humans. The homeobox shares significant homology with the MAT genes of yeast which encode gene-regulatory DNA binding proteins. These proteins interact with the cis-regulatory element in those genes which they control. These observations suggest a similar role for the homeotic proteins. In order to assess the possible DNA binding properties of the *Antp*-protein, an *Antp* polypeptide including the homeodomain, but lacking one third of N-terminal sequences, was overexpressed in a T7 vector in E.coli. The E.coli-made polypeptide was extensively purified and tested for sequence specific DNA binding. The results of immunoprecipitation and DNAse I footprinting to DNA sequences in the *Antp* first and second promoter and other homeotic genes are shown.

S15 68

PROCESSING (SPLICING AND 3'-END FORMATION) OF MESSENGER RNA PRECURSORS IN VITRO

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The removal of introns by splicing and the generation of the mature 3'ends by cleavage/polyadenylation are important steps in the processing of nuclear messenger RNA precursors (pre-mRNA). We are studying these reactions in vitro with synthetic RNA substrates and extracts or purified fractions from Hela cell nuclei. Splicing requires the ordered assembly of large multicomponent complexes (spliceosomes). Assembly of the complexes involves the binding of the major U-type snRNPs and additional protein factors to specific regions of the pre-mRNA. We have analyzed the requirements for this process by the use of mutant RNA substrates and by probing the U-snRNAs with RNase H and complementary oligonucleotides. Furthermore, we have separated four protein factors that are needed in conjunction with snRNPs for splicing components involved in the 3'-cleavage/polyadenylation of pre-mRNAs. This reaction also takes place in a large complex, the assembly of which requires several protein factors and probably a minor U-snRNP whose identification is one of the goals of our experiments.

S15 69

INITIATION OF PROTEIN SYNTHESIS IN YEAST S. CEREVIAIAE

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Over a dozen proteins are required for initiation of protein synthesis in eukaryotes. One of the first steps is the recognition of the cap structure at the 5'end of mRNA by the preinitiation complex. This complex then moves on the mRNA in the 3'direction until an AUG codon is encountered. There,initiation factors are released, elongation factors and the large ribosomal subunit join and protein synthesis begins. We have cloned the gene for the cap binding protein eIF-4E. We have shown that it is essential for viability. Furthermore conditional mutations using site-directed mutagenesis were generated. In a second approach we are identifying sequence requirements for reinitiation of protein synthesis on mRNA containing multiple reading frames. GCN4 mRNA contains four naturally occuring upstream reading frames. Evidence exists that inhibitory effects of upstream initiation codons can be suppressed by additional reading frames further upstream.This suppression is regulated in response to amino acid starvation

S15 70

IDENTIFICATION OF A GROWTH FACTOR- AND ONCOGENE-ACTIVATED S6 KINASE: UNMASKING OF A PHOSPHORYLATION CASCADE ?

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Experiments in vivo and in vitro support the notion that increased 40 S ribosomal protein S6 phosphorylation is a prerequisite for the activation of protein synthesis and cell growth. This idea has focused attention on the enzymes involved in regulating this event. Earlier we reported the presence of a stimulated S6 kinase in extracts of EGF-treated 3T3 cells whose activation paralleled in a kinetic and dose-dependent manner the phosphorylation of S6 observed in the intact cell. Subsequent studies have argued that a number of agents known to induce S6 phosphorylation all act through this same kinase. The enzyme loses activity during extraction unless phosphatase inhibitors are present, leading to the finding that the major S6 kinase phosphatase in cell extracts is a type 2A enzyme. Recently we succeeded in purifying the kinase to homogeneity. It is a Mr ≈ 70,000 protein that undergoes autophosphorylation and that phosphorylates all of the S6 peptides observed in vivo. Incubation of the pure kinase with phosphatase 2A abolishes all enzyme activity, arguing that the kinase is directly regulated by phosphorylation.

S15 71

BIOSYNTHESIS AND GLYCOSYLATION OF SUCRASE-ISOMALTASE AND DIPEPTIDYL PEPTIDASE IV AND DIFFERENTIATION OF HUMAN INTESTINAL EPITHELIAL CELLS (HT 29).

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The expression and mode of glycosylation of sucrase-isomaltase (S-I) and dipeptidyl peptidase IV (DPP IV) has been studied in undifferentiated and differentiated HT 29 cells by long pulse and pulse-chase experiments with ^{35}S-methionine, immunoprecipitation with Mabs and analysis by SDS-PAGE. The mode of glycosylation was analysed by treatment of immunoisolated proteins with endo H, endo F and trifluormethane sulfonic acid (TFMS). In differentiated HT 29 cells biosynthesis of these two markers was comparable to normal enterocytes. Expression of S-I in undifferentiated cells was at a very low level but processing kinetics were not significantly different to those of differentiated cells. The amount of DPP IV expressed was not appreciably greater in differentiated cells but a significant increase in Mr of the mature enzyme was observed. This size-difference has been shown to be partially due to increased O-linked glycosylation of DPP IV in differentiated HT 29 cells.

S16 72

NMR IMAGING: POSSIBILITIES AND APPLICATIONS

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During the last few years, NMR imaging of the 1-H nucleus has been introduced into clinical medicine. Based on an extraordinary soft tissue contrast, the free choice of the imaging plane, the lack of ionizing radiation, and the potential of qualifying and quantifying flow, it has obtained an even increasing important role in medical sectional imaging. Currently, its main indications are: cerebral lesions, mainly of the posterior fossa, the brain stem and the pituitary gland; spinal cord and vertebral disk disease; congenital and acquired cardiovascular pathology, especially when iodine-containing radiographic contrast agents have to be avoided; superficial joints where lesions of cartilage or ligaments can also be documented without conventional contrast agents; inflammatory and tumorous musculoskeletal pathology. The technical principles of NMR imaging as well as human anatomical and pathological applications will be demonstrated.

S16 73

NMR SPECTROSCOPY IN VIVO : POSSIBILITIES AND APPLICATIONS

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Nuclear Magnetic Resonance (NMR) Spectroscopy is a noninvasive method to identify quantitatively small and mobile molecules. Whereas until a few years ago, the measurements were restricted to volumes of around 1ccm in small sample tubes, enormous advances in NMR technology nowadays allow to observe metabolic processes directly (in vivo) in a selected volume of an object as large as a human being. The major restriction with NMR is its relatively low sensitivity, which sets the observation limit to concentrations of approximately 1millimolar. Todays investigations concentrate on the energy metabolism in various organs, where ATP, Phosphocreatine, Phosphate and intracellular pH can be measured on the P-31 nucleus. With H-1 NMR, lactate is observed along with other metabolites. C-13 NMR finally is able to monitor glycogen in liver and muscle, and selected metabolic pathways, if isotopically labeled precursors are used. The actual time resolution of these measurements is in the range of minutes.

S16 74

NMR STUDIES OF BODY FLUIDS : LIPOPROTEINS, ACUTE-PHASE PROTEINS, DRUGS, AND DISEASES

Peter J. Sadler, Department of Chemistry, Birkbeck College, University of London

We have analysed high resolution (400 and 500 MHz) ^{1}H NMR spectra from a variety of body fluids, e.g. plasma, urine, CSF, aqueous humour. Spectra can often be acquired within a few minutes from small volumes of sample (0.4 ml), non-destructively. Besides small molecules (natural and drug metabolites) we have studied mobile components of macromolecules (acute-phase glycoproteins, lipoproteins) and related the findings to disease conditions (e.g. diabetes, meningitis, cancer, organ damage). The technique provides new possibilities for study of metabolic biochemistry, and allows molecular interactions to be investigated.

S16 75

31-P NMR MAGNETIZATION TRANSFER IN THE RAT BRAIN: EFFECTS OF BARBITURATES AND CALCIUM ANTAGONISTS

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The rate constant (k) for the forward creatine kinase reaction (PCr+ADP+H$^+$-> ATP+Cr) was measured in the living rat brain by the NMR technique of magnetization transfer using a surface coil. Using an average T1(PCr) of 2.0±0.3 sec, the following k-values (mean±SE) have been found:

	Halothane	Pentothal	PN 200-110		
mg/kg ip	(2.5%)	40	0.5	2.5	5.0
k [sec^{-1}]	0.68	0.51	0.61	0.53	0.55
	±.03	±.03	±.04	±.04	±.04

Assuming that brain energy transfer via the creatine kinase reaction is closely coupled to energy production, as has been shown for the heart (Bittl & Ingwall, JBC 260 3512 (1985)), and since steady-state ATP levels remain constant, these results suggest that the calcium antagonist PN 200-110 reduces brain energy consumption. This effect of PN may be relevant for its cytoprotective activity, since reduction of infarct size in a rat stroke model has been found at similar doses (Stroke 17 1228 (1986)).

S16 76

IN VIVO ^{31}NMR PHOSPHOMONOESTER MEASUREMENTS CAN BE USED TO FOLLOW BRAIN DEVELOPMENT

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By using nuclear magnetic resonance (NMR) spectroscopy it is possible to measure in vivo and noninvasively brain phosphate containing compounds, such as phosphomonoesters (PME). With analytical in vitro ^{31}P NMR we showed that the PME peak of the rat brain contains phosphorylethanolamine (PE) and to a minor extent phosphorylcholine. PE is known to be a precursor of the phospholipid phosphatidylethanolamine (PhE), an important compound in brain membranes. In order to correlate in vivo NMR data with classical biochemical data we determined by ^{31}P NMR the age dependent changes of PME, observing the same animals during their development from 2 to 56 days. In parallel, PE and PhE were measured biochemically. We found a clear correlation between the age dependent decreases of PME and PE, and the aequivalent increase of PhE in the period of rapid myelinogenesis. These results indicate that in vivo ^{31}P PME measurements are useful to follow normal brain development.

Poster Session, Thursday

Anatomy, Histology, Embryology (AHE)

AHE 1

PRIMARY SENSORY NEURONS OF CHICK EMBRYO EXPRESS GLUTAMINE SYNTHETASE IN VITRO BUT NOT IN VIVO

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Glutamine synthetase (GS) catalyzes the ATP-dependent formation of glutamine from glutamate and ammonia.
To determine whether sensory neurons express GS, polyclonal antibodies raised to GS were provided by G. Tholey. Vibratome sections of DRG from chick embryos at E10, E18 or after hatching were free of any GS positive neurons. On the opposite, dissociated DRG cell cultures at E10 displayed numerous GS positive neurons. In neuron-enriched cultures, all the neurons were GS positive while in mixed DRG cell cultures, only 75% of the neurons were GS positive. Treatment of the cultures with actinomycin D for 45 h prevented GS-immunostaining of the neurons. No correlation was found between GS expression and ^{3}H-glutamine uptake by DRG cells. In conclusion, these data show that phenotypes of sensory neurons are influenced by environmental factors (SNF no. 3.397-86).

AHE 2

IS EXPRESSION OF CALBINDIN-IMMUNOREACTIVITY BY PRIMARY SENSORY NEURONS AFFECTED BY ENVIRONMENTAL FACTORS?

Philippe, E., Barakat, I. and Droz B.
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Immunoreactivity with antibodies to calbindin was expressed from E10 by 20% of sensory neurons in the chick dorsal root ganglia (DRG). The influence of target tissues on this phenotype was tested in the following conditions:
- When DRG were transplanted at E7 onto the chorioallantoic membrane of a host embryo, no immunoreactivity was observed 8 days later; but in DRG co-transplanted at E7 with myotubes, immunostaining was detected in about 14% of cell bodies. In contrast, in DRG transplanted alone at E12, about 20% of cell bodies were calbindin-positive, 8 days later.
- When dissociated DRG were cultured at E6, all the neurons were devoid of calbindin-immunostaining. However, addition of muscular extract promoted the appearance of calbindin-immunoreactive ganglion cells. When the muscle extract was added only after 3 days of culture, the % of calbindin immunoreactive neurons remained unchanged.
It is concluded that, 1) muscle factors prevent neuronal cell loss but this does not preferentially affect DRG cells committed to express calbindin, 2) initiation of calbindin expression is induced by muscle factors. (FNS No. 3.397-86).

AHE 3

PROSTAGLANDIN E_2 SPECIFIC BINDING SITES IN CHICKEN SPINAL CORD

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Prostaglandins (PGs) acts in the vicinity of their sites of biosynthesis. In the homogenate of the chicken spinal cord, which synthesized PGE_2, specific binding sites were identified. Indeed, PGE_2 saturable binding sites were recovered in the 17000g Pellet. The time required to reach equilibrium depends on temperature. Since at equilibrium, with 0.4 nM ^{3}H-PGE_2, 75% of the bound PGE_2 were displaced by a 1000 fold excess of unlabeled ligand, it is concluded that high affinity binding sites to PGE_2 are present in the chicken spinal cord.
(SNF N. 3.397.86).

AHE 4

IMMUNOCYTOCHEMICAL DETECTION OF PGD_2 ISOMERASE IN SUBPOPULATION OF CHICK DORSAL ROOT GANGLION (DRG) CELLS

VESIN, M.F.*, MOLLET, F.*, URADE, Y.$^{+}$ and HAYAISHI, O.$^{+}$,
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Biochemical studies have shown that DRG synthetize actively PGD_2. To determine the cell types which synthetize PGD_2 in DRG, antibody raised to rat brain PGD_2 isomerase were used under various conditions of processing; controls were performed with preimmune rabbit antiserum, immune serum to rat brain PGD_2 isomerase preabsorbed on cytosolic fraction of DRG or immune serum to isoenzyme PGD_2 isomerase from rat spleen. The immunoreactivity specific to rat brain PGD_2 isomerase was confined to a subpopulation including most of the small B ganglion cells and axons. In conclusion the cytosolic enzyme which catalyzes the final step of PGD_2 synthesis is exclusively detected in a defined population of primary sensory neurons.
(SNF No. 3397-86).

AHE 5

A NEURON-LIKE CELL TYPE, WHICH EXPRESSES NEUROFILAMENT AND NEURON SPECIFIC ENOLASE PROTEINS: MINISEGMENTS OF NEWBORN RAT OPTIC NERVE IN VITRO

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One of the simplest part of the central nervous system is the optic nerve, which in situ is free of neuronal cell bodies. In order to study the long term developmental course of progenitor and glial cells, we cultivated small explants, called minisegments (200-250 µm), of newborn rat optic nerves during various intervals of time. At 3 d up to 140 d in culture differentiated glial cells were present and showed the morphological and immunocytochemical properties of in situ glial cells. Surprisingly, after about 40 d in culture, a particular type of cell appeared. This cell type was negative for all tested glial cell markers but positive for the two specific neuronal markers mentioned. It can be suggested that optic nerves of newborn rat contain some neuroepithelial cells, which can give rise to either glial cells or neurons. The possible existence of such a common progenitor cell needs further investigations. A complete characterization of these "neuron-like" cells using electrophysiological techniques is in progress.
(Supp. Swiss MS and NSF 3.652.0.87).

AHE 6

DEVELOPMENT OF PROGENITOR AND GLIAL CELLS IN VITRO: MINISEGMENTS OF NEWBORN RAT OPTIC NERVE

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Optic nerves of newborn rat are mainly composed of unmyelinated axons, progenitor cells and astrocytes; neuronal cell bodies are absent, and oligodendrocytes and myelin sheaths appear only 6-7 days after birth. To study the developmental and functional potential of these different types of CNS cells in vitro, we cultured small pieces of optic nerves, called minisegments (200-250 µm) for various intervals of time (14 h up to 4 months). The procedure of cultivation was carried out in one phase or in two consecutive phases. First, minisegments were cultivated taking 4 conditions into account: (1) the tissue was not dissociated, (2) the size of minisegments must guarantee a permanent exchange of medium, (3) no use of substrate and (4) minisegments were kept under constant gyratory movement. Second, after various periods of gyratory cultivation, the minisegments were explanted onto collagen-coated (1%) petri dishes. An interdisciplinary characterization of the progenitor and glial cells using morphological, immunocytochemical and physiological techniques is established (Supp. Swiss MS and NSF 3.652.0.87).

AHE 7

BEHAVIORAL AND HIPPOCAMPAL MATURATION IN THE GUINEA PIG

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Identifying cerebral systems that still undergo maturation in a precocious species such as the guinea pig may permit to recognize behaviorally relevant brain circuitry. A total of 56 guinea pigs was investigated, 8 per age group, at the age of 5, 10, 20, 40, 80, 160, and 320 days. Behavioral testing of the animals included activity recording in an unfamiliar environment, and the learning of an escape response from electrical shock in a shuttle-box. For morphometry, recurrent mossy fiber collaterals below and above the granule cell layer were visualized by means of Timm's stain on parasagittal sections. The stained areas were measured along the septo-temporal axis by means of a video image analyzer, on 4 sections from the left and the right hemisphere. Behavioral testing revealed no differences between young and adult animals in the novel environment. Learning to escape from shock showed a rapid increase till the age of 40 days (around puberty) but leveled off afterwards. The plexus of recurrent mossy fibers showed a similar increase till to this age, but it appeared to grow afterwards in some animals. Hence, there is postnatal differentiation of limbic circuitry till puberty (coincident with the development of responses to shock), and, possibly, transadult differentiation as well.

AHE 8

CARBONIC ANHYDRASE (CA) IN DRG OF DEVELOPING CHICKEN DEPRIVED OF TARGET TISSUES

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Particular subpopulations of sensory neurons express CA activity in the DRG of chicken. The cytoenzymatic procedure of Hansson showed CA in a few ganglion cell bodies at E7. In order to determine whether the phenotypic expression of CA was influenced by target tissues, excision of one hindlimb at E6 or transplantation of DRG at E6 onto the chorioallantoic membrane of a host embryo were carried out. In both cases, DRG examined 5 days later were devoid of CA+ neurons. When hindlimb extirpation or DRG transplantation were performed at E12, about 25% neuronal cell bodies were CA+. These results suggest that the initiation of CA expression by sensory neurons requires interaction with target tissues; once the phenotype is expressed, CA activity is maintained in primary sensory neurons disconnected from targets. (SNF no. 3.397-86).

AHE 9

INFLUENCE OF VARIOUS TISSUES ON PRIMARY SENSORY NEURONS OF CHICK EMBRYOS IN COCULTURE OR CONDITIONED MEDIUM

Bossart E. (sponsor: Pr. G. Conti), Institut d'Histologie de l'Université de Fribourg, Fribourg.

Neuroblasts from chick embryo DRG were cultured at E6 in the F14 medium in the presence of NGF. After 5 days of culture, 11.6% of the seeded neurons survived and grew as clusters of small-sized cell bodies. No DRG cell expressed a calbindin-immunoreactivity.
In cocultures with DRG cells at E6, kidney cells produced a higher increase than myoblasts in: 1) the number of surviving neurons, 2) the neuronal cell volume . DRG cells cocultured at E6 with myoblasts or kidney cells displayed 3 or 20% of sensory neurons expressing calbindin immunoreactivity after 5 days of culture.
Conditioned medium obtained from cultures of kidney cells or myoblasts did not enhance the percentage of neuronal survival in DRG cell cultures at E6, but initiated the appearance of calbindin-immunoreactive neurons to a lesser extent than coculture.
The results suggest that cell-to-cell interactions, probably mediated by soluble factors, promote the expression of calbindin by neurons.
(Work performed at the Institut d'Histologie et d'Embryologie, Université de Lausanne; S.N.F. No. 3.397-0.86).

AHE 10

NON-DIRECTED AXONAL GROWTH ON EMBRYONIC BASAL LAMINA

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In the developing nervous system, nerve fibers always navigate along extracellular spaces at the basal margin of the neuroepithelium. The extracellular channels, which are rich in laminin are formed by the endfeet of the ventricular cells and by a basal lamina. The stereotyped location of axons at this site suggests that they provide favorable growth conditions and orientation cues for navigating axons. In order to study the role of the extracellular matrix and cell surface components on axonal navigation I have devised a procedure for isolating the basal surfaces of the embryonic avian retina and the pigment epithelium. Both preparations consist of a native, laminin-rich basal lamina with a surface area of up to 1 cm^2. The retinal basal lamian is in addition covered by a monolayer of ventricular endfeet that is removable by detegent treatment. Using these two substrates, the growth rates, densities and navigation of axons from neuronal explants of the central and peripheral nervous systems were studied. The growth rates and densities of axons on both these substrate preparations were shown to be identical; the removal of the ventricular endfeet from the retinal basal lamina had no effect on these parameters. The two substrates, however, provided better conditions for neurite outgrowth than collagen gels, laminin and fibronectin. Finally, on the prepared substrates, axons grow without orientational preference indicating that guidance cues are not imprinted in basal laminae.

Pharmacology, Toxicology (PHA)

PHA 11

DRUG BINDING STUDIES WITH THE S- AND F-FORMS OF HUMAN ALPHA-1-ACID GLYCOPROTEIN VARIANTS BY MULTICOMPARTMENTAL EQUILIBRIUM DIALYSIS

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By this procedure (Bickel et al., J. Pharm. Sci. 76 (1987) 68-74), the binding of several drugs, i.e. amitriptyline, imipramine, clomipramine and their demethylated metabolites, and of trimipramine, maprotiline, methadone, thioridazine and mephenytoin to the S- and F-forms of alpha-1-acid glycoprotein (S-, resp. F-AAG) has been studied. The genetically determined forms of AAG, S- and F-AAG, have been isolated by isoelectric focusing from commercially available AAG. The basic drugs bind preferentially to S-AAG rather than to F-AAG:this selectivity of binding is the highest for methadone, the lowest for thioridazine and absent for the acidic drug mephenytoin. It is more pronounced for the secondary amines than for the corresponding tertiary amines. This finding suggests a genetic factor contributing to the interindividual differences in the binding of drugs to AAG.

PHA 12

IDENTIFICATION OF MUTANT ALLELES OF THE P450db1 GENE ASSOCIATED WITH GENETICALLY DEFICIENT METABOLISM OF DEBRISOQUINE AND OTHER DRUGS

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The "debrisoquine polymorphism" is a clinically important genetic defect of drug metabolism affecting 5-10% of individuals. A full length cDNA for human cytochrome P450db1, the deficient enzyme, has recently been cloned. We have examined DNA from normal and defective (PM) individuals using the db1 cDNA as hybridization probe. Digestion with XbaI endonuclease revealed two restriction fragment length polymorphisms (RFLPs) associated with the PM-phenotype. Segregation of these fragments in 6 families confirmed the identification of two independant mutated alleles of the P450db1 gene. 75% of defective individuals had one or both polymorphic XbaI fragments. A third, but probably additional mutant alleles not detected by RFLPs must be present in the population.

PHA 13

EFFECTS OF THE ANTIDEPRESSANT DRUG DESIPRAMINE (DMI) ON MEMBRANE FLUIDITY OF CULTURED RAT BRAIN CELLS

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Effects of the antidepressant drug DMI on membrane fluidity of cultured astrocytes and Roc-1 cells (oligodendrocytesXC6) were investigated by means of a fluorescence polarization technique using anthroyloxy-stearic (nAS) or -palmitic (2AP) acids as markers. Cells were suspended in Hank's and apparent fluorescence anisotropy rG, inversely correlated to fluidity, analyzed at 37°C in control cells and in cells following single dose or multiple dose exposure to 5 µM DMI. rG values (6 and 12AS) were identical for both cells. The presence of DMI (6AS) led to a small, non-significant increase in anisotropy and (12AS) to a significant (*) decrease of anisotropy to the same extent in both cell types. Following 7 doses of 5 µM DMI rG values were analyzed after removal of the drug with the markers 2AP, 6-,7-,9-,12AS. Data were expressed in % of controls. The rG distribution in astrocytes was 92*,94*,92*, 91* and 93*% and in Roc cells 106*,109*,107*,105 and 101 %(*:p<0.05). Data indicate the presence of DMI is fluidizing the deeper, hydrophobic layer of the membranes, whereas chronic exposure leads to different modifications of the rG pattern which might be specific for different cell membranes.

PHA 14

MODEL SYSTEM FOR DETECTION OF TERATOGENS: SERUM-FREE PRIMARY CULTURES OF FETAL CHICK NEURONAL RETINA, BRAIN AND MENINGUS CELLS.

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In order to develop an in vitro screening system for detection of teratogens fetal chick (stage 28) retina, brain and, for comparison, meningus cells were chosen in order to test for subchronic drug effects. Cells were isolated by mechanical dissocciation (viability: 85-95%). Monolayer cultures were produced by plating cells in microtiter wells (50 ul of serum-free medium) coated with poly-D-lysine and collagen type I. After 1 day of incubation another 50 ul serum-free medium was added containing various concentrations of the test substances. The survival of the monolayer cultures was 14 and 20 days, for retinal and brain cels, respectively. Degree of differentiation of the nerve cells and the proliferation rate of the glia cels were directly correlated with the poly-lysine concentration. In retinal cultures part of the glia cells were found to differentiate into pigment containing (pigment-epithelial-like) cells. By electron microscope these findings could be confirmed. Also the formation of synapses could be observed. Drug effects were tested by measuring the endpoints cytotoxicity and nerve cell differentiation (morphology, pH change of the medium, presence of MAP_2 antigens). Experiments with colchicine, $CdCl_2$, EDTA-$Ca^{++}Na_2$ revealed dose-response curves for each chemical according their teratogenic potential.

PHA 15

BENZYL ALCOHOL IMPAIRS Ni-MEDIATED INHIBITION OF ADENYLATE CYCLASE ACTIVITY IN RAT STRIATAL MEMBRANES.

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Benzyl alcohol (BA) has been shown to increase membrane fluidity in vitro and to modulate adenylate cyclase (AC) activity in several tissues. In rat striatal membranes, BA (15-70mM) potentiates the stimulation of the enzyme by guanylylimidodiphosphate [Gpp(NH)p], whereas the responses to forskolin and calmodulin are inhibited. This study was carried out to determine the mode of action of BA on Gpp(NH)p-dependent AC activity. BA increased the stimulatory effect of Gpp(NH)p on AC in membranes preloaded at 30°C with the nucleotide, indicating that the effect occurs at a step beyond Ns activation. The effect of BA on Ns-dependent activation was lost in the lubrol-solubilized enzyme, suggesting that an intact bilayer is required for this effect. When the assay was carried out at 20°C or in the presence of forskolin, inhibition of AC by Gpp(NH)p was observed. This inhibition was lost in the presence of BA (15mM) and activation was actually observed with 50mM BA. Activation by BA also occured in membranes preloaded with Gpp(NH)p under inhibitory conditions (20°C). Our results suggest that the expression of Ns (coupling to the catalytic unit) is favoured over that of Ni when membrane fluidity is increased by BA.

PHA 16

EFFECTS OF DEFEAT ON FRONTAL POLE (FP) DOPAMINE METABOLITES IN DBA/2 AND C57BL/6 MICE

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Frontal pole (FP) dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were determined by HPLC after a nonaggressive confrontation (control) and 1 min, 20 min, 2 hrs, and 24 hrs after a defeat (30 bites). FP DOPAC was enhanced 20 min after defeat in both mouse strains (DBA: .10±.02 (ng/mg tissue wet weight) n=9 to .20±.05, n=5, C57: .09±.02, n=9 to .19±.08, n=5, $p<0.01$, ANOVA), while there were no changes 1 min, 2 hrs and 24 hrs after defeat. FP HVA was enhanced 20 min after defeat in both strains of mice (DBA:.09±.02, n=9 to .13±.04, n=5, C57: .07±.03, n=9 to .10±.01, n=5, $p<0.01$) and remained elevated in DBA but not in C57 mice 2 hrs after defeat (DBA: .15±.03, n=5, C57:.07±.01, n=5). Our results indicate that FP is involved in defeat stress and a strain specific recovery of the defeat effects in FP.

PHA 17

STUDIES OF ANTIDEPRESSANT DRUG ACTIONS ON ISOLATED MEMBRANE VESICLES OF CULTURED CELLS

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Chronic exposure of four different cell culture models to the antidepressant drug desipramine (DMI) led to decreases in the number of functional β-adrenoceptors and to changes in the cellular phospholipid(PL)-content and -composition. To investigate whether these changes are related, it became mandatory to study the drug effects on the plasma membrane. The isolation of plasma membrane vesicles (PMV) was achieved by a modification of the method of R.E.Scott (Science 194, 743, 1976): Vesiculation was induced by incubating the cell monolayers with 25 mM formaldehyde and 2 mM dithiotreitol. PMV could be washed away from the cells. A 6-9 fold enrichment of the membrane marker enzyme 5'-nucleotidase and a 20-30 fold loss of the lysosomal marker β-glucosidase proved a sufficient purification of the plasma membrane. This preparations allowed to determine the PL-composition, the cholesterol content and the fluidity in the membrane as the relevant compartment of antidepressant drug effect.

PHA 18

IMPAIRED MOTOR COORDINATION AFTER SINGLE DOSES OF THE BENZODIAZEPINE TRIAZOLAM IN ELDERLY VOLUNTEERS (Eld).

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Disproportionate effects of benzodiazepines (bdz) in Eld may be due to decreased elimination and/or hypersensitivity of the CNS. To test these alternatives triazolam (tr) was used as model drug. Doses of 0.25 mg p.o. were given to 10 Eld (63 to 80 y) and 8 controls (Co, 29 to 60 y, referred for a check up). Two h after dosing, median free tr plasma concentrations (capillary GC-ECD; equilibrium dialysis) were 0.42 and 0.40 ng/ml; $t_{½e}$ were 3.8 and 4.1 h, in Eld and Co. After 2 h significant effects were observed: critical flicker fusion showed 6 and 10%, digit symbol substitution test 17 and 18%, and pursuit rotor (PR), optimized for used in Eld, 47 and 24% change in Eld and Co, respectively. Only PR showed a difference between Eld and Co ($p<0.05$). Afer 6 h all measures had returned to baseline. Conclusion: Eld exhibit significantly more impairment in psychomotor performance, increasing the risk of accidents after intake of tr and possibly other bdz.

PHA 19

EFFECT OF PRENATAL NICOTINE EXPOSURE TO CENTRAL NICOTINE BINDING SITES

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The effect of prenatal nicotine exposure from gestational day 12 - 19 was investigated in six day old rat pubs. Alzet minipumps containing either nicotine bitartrate or tartaric acid were implanted subcutaneously to Long Evans dams. ^{3}H-nicotine binding sites were studied in brains of offsprings by in vitro autoradiography (Odermatt et al. Experientia 43 (87)). Quantitative densitometric measurements were done with a computer assisted Leitz-ASBA morphometric system.
Nicotine treatment was found to increase binding sites in substantia nigra pars compacta, while in the lateral geniculate nucleus a decrease was observed. Additional preliminary measurements were also done in neocortex, caudatus putamen and in thalamus. So far no significant changes have been observed in these brain regions.

PHA 20

CHARACTERIZATION OF BZR LIGANDS IN RATS IN A PALATABLE FOOD CONSUMPTION PARADIGM

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A large body of recent literature has clearly demonstrated that compounds differentially affect consumption of a palatable diet in non-deprived rats depending upon intrinsic efficacy at the benzodiazepine receptor (BZR) complex. Compounds characterized in other test situations as acting predominantly as BZR agonists induce hyperphagia, those classified as acting predominantly as BZR inverse agonists produce hypophagia, and those exhibiting predominantly a BZR antagonistic profile have minimal effect on palatable food intake when given alone, but reduce the effects of BZR agonists and BZR inverse agonists. In a palatable food consumption paradigm, diazepam acted as a BZR agonist, flumazenil as a BZR antagonist with a weak BZR agonistic component, and both Ro 15-4513 and Ro 19-4603 exhibited a BZR inverse agonistic profile. These results are consistent with previously obtained biological data.

PHA 21

BRAIN GLUCOCORTICOID RECEPTORS (GR) &CHOLINERGIC FUNCTION: EFFECTS OF FOOD RESTRICTION AND AGING

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Male Sprague Dawley rats were allocated into 4 groups. Group A: 3 month-old rats fed ad libitum. Groups B, C, D: 24-25 month-old rats, fed from 6 months of age the following diets: Group B: diet ad lib; Group C: fed 70% of the mean dietary intake of group B; Group D: fasted for 2 non-consecutive days per week, else ad lib. GR binding [Bmax] was determined in hippocampus (HC), amygdala (AM) and hypothalamus (HT). Groups B and C showed an age-related decline in GR Bmax in HC and AM, while group D values in HC remained similar to those of group A. Diet and age had no effect on GR in HT. Cholinergic function was evaluated by release and contents of striatal acetylcholine (ACh) and choline. Tissue contents of ACh were similar in all groups; choline levels were, however, decreased in group B. Diet and age influenced neither resting nor evoked ACh and choline release. These results suggest that fasting may delay the age-related decline in hippocampal glucocorticoid receptors and food restriction maintain striatal choline levels.

PHA 22

DETERMINATION OF AROMATASE-ACTIVITY IN THE DEVELOPING RAT BRAIN

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Brain steroid aromatase, which converts androgens to physiologically active metabolites such as estrogens, is known to be important in behavioural and sexual differentiation of vertebrates. Furthermore, aromatization is known to take place only in very restricted areas of the brain, such as hypothalamus, amygdala and preoptic area. To obtain more detailed data on the specific localization and change in aromatase-activity of the developing male and female rat brain, we have investigated this enzyme activity in Long Evans rats during different periods of development, i.e. pre- and postnatal up to adulthood. This enzyme is determined through the evaluation of the conversion of 1β-^{3}H-androstenedione to estrone. With a modification of the method of Thompson & Siiteri we measured the amount of tritiated water formed during one hour of incubation after separation of the steroids by Sepralyte-C_{18}-columns.

PHA 23

IN SITU HYBRIDIZATION HISTOCHEMISTRY: A TOOL TO STUDY CELLS EXPRESSING GABA/BENZODIAZEPINE RECEPTORS AND CELLS AFFECTED BY GABA/BENZODIAZEPINE RECEPTOR LIGANDS

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^{35}S-Labelled RNA probes complementary to the mRNAs of the two constituent proteins of the GABA/benzodiazepine receptor (α- and β-subunit) have been used to determine, by in situ hybridization histochemistry, the cells that express the receptor. Cells which showed a strong hybridization signal were mainly distributed in cerebral cortex, hippocampus, thalamus and cerebellum in accordance with the immunocytochemical distribution of the GABA/benzodiazepine receptor protein.
In order to study whether all these cells are functionally affected by GABA/benzodiazepine receptor ligands, the induction of the protooncogene c-fos was studied as marker of neuronal activity. In animals which experienced transient seizures following treatment with the inverse agonist DMCM, c-fos induction was determined in brain sections by in situ hybridization. Receptor activation mainly affected neurons in cerebral cortex and gyrus dentatus.

PHA 24

NOVELTY-INDUCED ANALGESIA AND LEARNING IN SIX STRAINS OF RATS

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Exposure of Wistar rats to a novel environment is followed by a mild analgesia (Siegfried et al. Behav. Neurosci. 101,436, 1987). We measured the tail-flick latency (TFL) in 6 genetically distinct strains of rats immediately before and 2min. after exposure to either an openfield or the homecage and compared it to openfield behavior (locomotor activity, rearing) and to shuttlebox avoidance. Whereas homecage-exposure induced a significant TFL elevation in only 1 strain, in 5 out of the 6 strains a significant TFL increase became apparent subsequent to the openfield-exposure. The conditioned avoidance response (%) was RHA/Verh(71), Lewis(63), WKY(55), Fawn Hooded(34), Wistar(24) and RLA/Verh(1), respectively. The extent of the net novelty-induced analgesia (openfield - homecage) [RHA/Verh +1.01, Lewis +.91, Fawn Hooded +.88, WKY +.65, Wistar +.61, RLA/Verh +.46sec.] was not related to openfield behavior. However, a correlation ($r=+0.619; 2p\ 0.05$) between the net novelty-induced analgesia and avoidance performance was evident. The participation of hippocampal structures in the genotype-assessed relationship between novelty-induced analgesia and learning will be discussed.

PHA 25

CENTRAL HIGH, BUT NOT LOW AFFINITY NEUROTENSIN (NT) BINDING SITES ARE IMPLICATED IN THE NT-INDUCED HYPOTHERMIA IN RATS

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High and low affinity NT binding sites exist in rat brain. To examine their physiological significance, we compared the interaction of NT, a pentapeptidic analog (P) and levocabastine (L) with high (labeled with 125-I-NT) and low affinity (labeled with 3H-NT) NT binding sites in rat brain membranes. In vivo, we studied the drug-effects on rat body temperature after i.c.v. and i.v. injections. IC_{50} (nM) of the drugs on 3H-NT and 125-I-NT binding were: NT: 20 and 2.27; P: 30,000 and 175. On 125-I-NT binding, P displayed a Hill Coefficient of 0.645 (compared to 1.17 for NT), indicating a two sites system with IC_{50} of 13 and 683 nM. L displaced 80% and 40% of 3H- and 125-I-NT binding (IC_{50}: 700 and 25 nM). After i.c.v. injection, NT and P produced hypothermia (ED_{50}: 4.2 and 7.1 nmoles/rat). Both peptides were also active i.v. L did not produce hypothermia in any case. In conclusion, P shows strong selectivity for high affinity NT binding sites. Thus, the high, and not the low affinity NT sites may be of physiological relevance.

PHA 26

PHARMACOLOGY AND BIOCHEMISTRY OF TWO NOVEL PRESYNAPTIC DOPAMINE (DA) RECEPTOR ANTAGONISTS IN RAT BRAIN

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(+)-UH 232 (UH) and (+)-AJ 76 (AJ) (Prof. Carlsson, Goeteborg) are aminotetralins interacting preferentially with brain DA autoreceptors. Such drugs could constitute novel antidepressants or neuroleptics (NL). They were examined in biochemical, receptor binding and behavioral tests. In vitro, UH and AJ interacted markedly with D2 receptors. On striatal slices, the high ratio release of 3H-DA/14C-ACh indicated preferential blockade of pre- vs postsynaptic DA receptors. In vivo, UH resembled atypical NL: it inhibited 3H-spiperone binding in DA areas, with a highly selectivity for hippocampal DA receptors; it increased DA turnover in striatum, and produced mild behavioral stimulation at low doses, and sedation at higher. AJ produced multiphasic dose-response curves of 3H-spiperone binding. In striatum, in particular, AJ increased the binding presenting analogy with bupropion. In the other tests, it resembled UH. In conclusion, UH and AJ are preferential DA autoreceptor blockers, but some qualitative differences are suggestive for distinct therapeutic use.

PHA 27

ETHANOL POTENTIATES THE INCREASE IN cAMP LEVELS ELICITED BY VIP AND/OR NORADRENALINE IN MOUSE CEREBRAL CORTICAL SLICES

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Among several effects, ethanol (EtOH) affects membrane fluidity and lipid-protein interactions. As proteins are influenced by surrounding lipids, the activity of membrane-bound enzymes such as adenylate cyclase (AC) could be modulated by EtOH, as shown in potentiating,at toxic concentrations, the hormone- or neurotransmitter-effects. We have also found that EtOH potentiates in a dose-dependent manner (EC_{50}=100mM) cAMP production elicited by VIP, already perceptibly at 70mM,without affecting basal cAMP levels (up to 400 mM).Propanol produces a similar potentiation,whereas methanol (200mM) was inactive. Butanol (200mM) displays toxic effects. The potentiating effect of EtOH is similar for peptide- (VIP) or monoamine-(noradrenaline (NA)) stimulated cAMP formation, suggesting a primary action at a level different from the receptor recognition site. Finally, we have observed that EtOH potentiates the synergistic interaction between VIP and NA in stimulating cAMP formation,the synergism being mediated by α_1-adrenoreceptor.

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PHA 28

NEUROTRANSMITTER-MEDIATED ^{3}H-GLYCOGEN HYDROLYSIS IN PRIMARY CULTURES OF MOUSE ASTROCYTES.

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Primary astroglial cultures from perinatal mouse cerebral cortex have been established; GFAP-positive cells ranged between 85-90%. Neurotransmitter-induced glycogenolysis was monitored in these cultures. We used 6-^{3}H-D-glucose and 6-^{3}H-D-galactose as substrates for ^{3}H-glycogen synthesis. After a 24 hour incubation with these precursors, various neuroactive agents were added for 30 minutes and their effect on ^{3}H-glycogen levels was assessed. VIP, norepinephrine, adenosine and dopamine promoted a marked ^{3}H-glycogen hydrolysis. Dose-response curves for VIP and NE yielded EC_{50} of $2,5.10^{-8}$M and $1,5.10^{-8}$M respectively. Control cultures from leptomeningeal cells, the major contaminant type in astroglial cultures, did not demonstrate significant glycogenolytic response to these neurotransmitters. Other neuroactive substances, such as excitatory amino-acids and acetylcholine were not glycogenolytic. These observations suggest that certains neurotransmitters can mobilise energy substrates from astrocytes, which constitute the major glycogen storage compartment of the brain.

PHA 29

CHOLINE ACETYLTRANSFERASE AND NERVE GROWTH FACTOR AFTER LOW LEAD EXPOSURE

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The effect of low lead exposure on choline acetyltransferase (ChAT) activity, has not yet been sufficiently elucidated. In our experiment, lead acetate was given to rat dams (Long Evans) in the drinking water (Widmer et al., Neuroscience, Vol. 22 Suppl., 87). ChAT activity was measured in the offspring at postnatal day 28.
We found that ChAT activity was significantly increased in hippocampus of lead treated animals, but was unaffected in controls. No difference was detectable in occipital cortex. A very similar pattern is seen for total protein content. We also measured nerve growth factor levels in these two brain regions and again no difference was observed in cortex, whereas in hippocampus we found a slight increase in lead treated animals.

PHA 30

CHARACTERIZATION OF SOCIAL CONFLICT ANALGESIA IN MICE

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Exposure of DBA/2 mice to attack bites by a dominant C57BL/6 mouse induced analgesia, measured with the tail-flick test. Low-intensity analgesia (LIA), found after 7 bites, dissipated within 10 min, and was naloxone (NX, 10 mg/kg) insensitive, but completely prevented by the alkylating opiate antagonist β-chlornaltrexamine (CNA, 5 mg/kg). High-intensity analgesia (HIA), found after 50 bites, lasted up to 60 min, and was partially prevented by pretreatment with NX, or CNA. Participation of endogenous opioids in social conflict analgesia was further suggested by a decreased β-endorphin level in the periaqueductal grey, as well as by the simultaneous recovery of opioid binding and HIA after receptor blockade by CNA. Development of LIA was associated with the display of defensive upright postures, whereas HIA delayed the occurrence of panic escape reactions. Preexposure of test mice to a nonaggressive C57BL/6 opponent prevented LIA 24 h later, while preexposure to 50 bites changed LIA 24 h later into a more pronounced analgesic response (long-term analgesia). The effects of aggressive and nonaggressive experience on LIA were absent in animals preexposed under NX. In conclusion, endogenous opioids play an important role in social conflict analgesia and in its modification by previous experience.

PHA 31

THE MOLECULAR BASIS OF NEURONAL RECOGNITION IN THE DEVELOPING LIMBIC SYSTEM.

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During brain development, neurons make connections with one another with great precision. The molecular basis of this phenomenon is a major subject of investigation in neurobiology. So far, molecules have been described in lower vertebrates and in invertebrates, that are involved in processes like neuronal migration and axon fasciculation. Clearly, these developmental steps are a necessary, but not sufficient condition for the setup of specific connections in the brain. The aim of our work is to identify surface molecules, expressed on functionally related groups of neurons and axons, that could mediate axon-target recognition in the developing CNS of mammals. Here we show that monoclonal antibodies directed against the Limbic System Associated Membrane Protein (Levitt,1984, 1986) specifically block axon-target recognition in the septohippocampal pathway in vitro, without affecting neuronal survival and axon growth in itself. These observations represent the first experimental confirmation for the chemoaffinity hypothesis of axon growth (Sperry,1963) in the mammalian brain.

PHA 32

REGULATION OF β-ADRENERGIC RESPONSIVENESS DURING ERYTHROID CELL MATURATION

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Reticulocyte(R) maturation is accompanied by a rapid loss of β-receptor(β-R)-coupled adenylate cyclase(AC) activity. On the other hand, Friend erytroleukemic cells (F-cells) enhance their β-adrenergic responsiveness during DMSO-induced differentiation into normoblasts. The mechanisms regulating these changes in hormonal sensitivity are still unknown. We have used R-F-cell-hybrids as a model system to probe the regulation of the β-adrenergic system during differentiation. Hybrids with functionally coupled β-R from R and AC from F-cells were prepared by fusion of undifferentiated F-cells (devoid of β-R) with R (NEM-inactivated AC). 50 % of the initial AC-activity in these cells was lost within 18 h after fusion. Under identical conditions (β-R and AC from R and F-cells, respectively) hybrids between differentiated F-cells and R showed receptor-coupled AC activity that increased 4 fold within 18 h after fusion. These results suggest that cytosolic factors rather than endogenous properties of the β-R-cyclase system regulate hormonal responsiveness during cell maturation.

PHA 33

EFFECT OF EXTRA- AND INTRACELLULAR pH ON THE RELEASE OF CATECHOLAMINES FROM RAT ADRENAL: INTERACTIONS WITH Ca++.

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Left adrenal glands of male Wistar rats were perfused from the venous side with oxygenated Ca++ free Krebs bicarbonate sol. at 0.2 ml/min at 36°C. Pulse stimulation of glands (30 sec) with Ca++ free low pH solution (buffered with 20 mM HEPES) slightly increased adrenaline (ADR) at pH 6.5 but not noradrenaline secretion. In the presence of Ca++ (1 mM) ADR output significantly increased between pH 5-6; pH 6-7 ADR output gradually returned to control levels. Pulse acidification of intracellular medium with a CO_2 saturated solution significantly stimulated ADR output in the absence of Ca++. Ca++ (10^{-7} to 10^{-5}) did not affect the CO_2 stimulation on the ADR release. However, Ca++ (10^{-4} to 10^{-3} M) did increase CO_2 stimulated ADR release. Neomycine 10^{-3} M (N), an inhibitor of phospholipase C (PLC) significantly decreased (70%) CO_2 stimulation. Stimulation of ADR release with acetyl-B-methylcholin was also noncompetitively blocked by N (10^{-3}). These results indicate that, lowering extracellular pH increases ADR secretion by gradually depolirizing cell membrane betwen pH 6-7. Lowering intracellular pH might increase ADR secretion through activation of PLC as the M_1 receptor does. (Supported by S.N.S.F. Gr. Nr. 3.168.0.85).

PHA 34

ADENOSINE RELEASE AND ACTIVATION OF GLYCOGENOLYSIS IN RABBIT NERVE FIBRES

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The fluorescence of NAD(P)H in desheathed vagus nerve was recorded at 450 nm with excitation at 366 nm. In resting nerve the fluorescence was not much changed when glucose was added, but markedly decreased when the glucose concentration was reduced; further, a small increase in fluorescence was observed after addition of Ca. Addition of adenosine produced an increase in fluorescence with an EC_{50} of 75 uM, forskolin 4 uM had a similar effect. As described by Landowne & Ritchie (*J. Physiol.* **212**, 483, 1971), an increase in fluorescence is also produced by electrical stimulation. We found that the activity induced increase was dependent on external Ca or glucose, and reduced by the adenosine antagonist 8-phenyl-theophylline. Taken together these results suggest that adenosine released during activity from the axons (Maire, Medilanski & Straub, *J. Physiol.* **357**, 67, 1984) may be involved in the increase in fluorescence by stimulating glycogenolysis and thereby increasing glycolyis.

PHA 35

CARBACHOL, HISTAMINE, AND BRADYKININ INCREASE INOSITOLPHOSPHATES IN RABBIT NERVE FIBRES

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Phosphoinositides were radiolabelled by incubating desheathed vagus nerves in myo-2-^{3}H inositol; after 2 hr LiCl (10 mM) was added to trap inositol-mono-phosphate (IP_1), and after a further hr, the compounds to be tested. Next, the preparations were transferred to ice-cold perchloric acid, sonicated, the inositols extracted, separated by HPLC, and counted. After application for 1 hr of either carbachol (1 mM), or acetylcholine (10 uM) plus eserine (30 uM), or arecoline (1 mM), or oxotremorin (1 mM) the IP_1 content increased by 200 % compared to untreated controls, pilocarpine (1 mM) caused an increase of 100 %. Application of carbachol for 2 min increased the content in inositol-bis-phosphate (IP_2) by 300 %, while the effect on IP_1 was not yet detectable. The effect of carbachol was blocked by atropine, but not by hexamethonium. An increase of 200 % was found with histamine (0.1 mM) and an increase of 300 % with bradykinin (10 uM). Adenosine, 2-chloro-adenosine (0.5 mM), and serotonine (1 mM) had no effect. The results show the presence of muscarinic, histaminergic, and bradykinin receptors in peripheral nerve fibres and suggest that these receptors can activate the phospholipase C system.

PHA 36

ELECTRICAL EVENTS IN RABBIT AORTA

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The rabbit aorta is widely used as a blood vessel for contractile measurements in in vitro investigations. Recent studies (Cauvin et al., J. Cardiovasc. Pharmacol. 6, S630-S638, 1984) suggest that contraction of rabbit aorta in response to noradrenaline occurs in the absence of changes in membrane potential. The following results demonstrate that this is not the case. The resting membrane potential of the vascular smooth muscle cells of the rabbit aorta was found to be in the range of -60 to -50mV and varies more than, for instance, that of the rabbit main pulmonary artery (-63 to -57mV). Noradrenaline and the α_1-agonist methoxamine produced a concentration-dependent decrease in membrane potential with a maximum depolarization by 12-18mV and contractions of similar maximum for both agonists. There were only slight contractions in response to the α_2-agonist B-HT 920 and virtually no change in membrane potential. Maximal depolarization to angiotensin II was by 4-8mV and the efficacy of angiotensin II to contract the aortic strips was two thirds that of noradrenaline or methoxamine. It is concluded that stimulation of α_1-adrenoceptors and angiotensin receptors results in depolarization of the vascular smooth muscle cells of the rabbit aorta. The ineffectiveness of the α_2-agonist B-HT 920 is explained by the lack of α_2-adrenoceptors in rabbit aorta.

PHA 37

SELECTIVITY OF CALCIUM AGONISTS IN HEART MUSCLE

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Calcium agonists promote Ca++influx in a variety of cells. In order to clarify their direct selective action on slow Ca++ channels experiments in isolated heart muscle preparations were performed. The dihydropyridine derivatives (+)-(S)-202-791 (Hof et al., J. Cardiovasc. Pharmacol. 7 (1985) 689), BAY K8644 and CGP 28392 (Kevin et al., Life Sci. 37 (1985) 1271) all restored electromechanical activity of quiescent partially depolarized ([K+]o 22 mM) guinea-pig papillary muscles. The action of (+)-(S)-202-791 was not altered by propranolol, prazosine and cimetidine but was antagonized by its Ca++ antagonistic enantiomer (-)-(R)-202-791. The actions of BAY K8644 and CGP 28392 were not altered by carbamylcholine, which lowers elevated cAMP levels but were blocked by the organic Ca++ antagonists darodipine and verapamil respectively. These results suggest that (+)-(S)-202-791, BAY K8644 and CGP 28392 activate Ca++ entry in heart muscle by selective direct modulation of slow Ca++ channels without involvement of cAMP.

PHA 38

CHARACTERIZATION OF CALCIUM CHANNELS IN VASCULAR SMOOTH MUSCLE CELLS

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Influx of calcium into smooth muscle is regulated by two pathways that can be distinguished pharmacologicaly: potential (POC) and receptor operated calcium channels (ROC).In our aortic smooth muscle cells, opening of the POC by depolarization with 55 mM KCl yields a two-fold increase in $^{45}Ca^{2+}$ uptake. This effect is blocked by organic calcium entry blockers with potencies similar to the inhibition of KCl induced contraction of the aorta.

ROC's were studied with the agonists vasopressin, angiotensin II and ATP. They increase the initial $^{45}Ca^{2+}$ influx in a concentration-dependent manner. However organic calcium entry blockers (diltiazem, dihydropyridines, verapamil) don't affect this increase. Inorganic blockers (La^{3+}, Co^{2+}, Cd^{2+}; 1mM) were able to block ROC's as well as POC's. $Cd^{2+}$was about ten times more selective for POC's.Mg^{2+} displaced Ca^{2+} in both, POC and ROC competitively. This shows that these cultured smooth muscle cells have two pathways for regulating $^{45}Ca^{2+}$influx with different pharmacological sensitivities.

Cell and Molecular Biology (CMB)

CMB 39

STUDIES OF THE CONFORMATION OF d-$(AAAAATTTTT)_2$ USING TWO-DIMENSIONAL ^1H NUCLEAR MAGNETIC RESONANCE

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The presence of (dA)n tracts give DNA unusual structural properties which influence e.g. bending of DNA and its ability to reconstitute nucleosomes. Thus the three-dimensional structure of the decadeoxynucleotide d-$(AAAAATTTTT)_2$ was investigated in solution by NMR. Phase-sensitive 2D NMR experiments at 500 MHz were used to obtain sequence-specific resonance assignments. Conformational constraints were collected from ^1H NOESY spectra, and from quantitative simulations of the cross peaks in ^1H COSY spectra. At T=20°C, 0.05M phosphate, 0.1M NaCl, pH=7.0, the decanucleotide duplex adopts overall a B-DNA-type conformation. Intra- and interstrand NOE's between adenine-2H and deoxyribose-1'H are compatible with pronounced propeller twisting, as was also observed in single crystal structures of other DNA-fragments containing (dA)n tracts. NOE data and the spin-spin coupling constants showed, that the deoxyribose pucker for the nucleotides T6 and T7 is different from that of A1 to A5, with pseudorotation phase angles P in the range 100° to 130° (04'-endo to C1'-exo) for the thymidines and 150° to 180° (≈C2'-endo) for the adenosines.

CMB 40

EVOLUTION OF THE GENE, ENCODING THE Ca^{2+}BINDING PARVALBUMIN

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Recently, we described the intron/exon organization of the rat parvalbumin gene and established its relationship to other genes for Ca^{2+}-binding proteins. In addition, we localized the human gene on chromosome 22 by the somatic cell hybrid technique (Berchtold et al. J.B.C. 262, 8696, 1987). To analyze the evolution of the parvalbumin gene a human parvalbumin clone was isolated from a chromosome 22 specific library. Four of five expected exons, including 400 bp upstream of the putative transcription start site and 90% of the coding region were found within a single 7.5 Kb Eco Rl fragment. Splice site positions with respect to the amino acid sequence were identical in the human and rat parvalbumin gene. The coding region has 92% sequence homology. Only 9 conservative aminoacid replacements could be predicted from exon sequencing. One stretch of the human promoter (-79 to -110) is identical to the rat counterpart. This region has a high homology to the promoter of the gene for myosin light chain 3F (Nabeshima et al. Nature 308, 333, 1984). This gene is expressed in fast contracting/relaxing muscle fibres (anaerobic, type IIb), the cell type which exhibits also highest parvalbumin expression. We conclude that this promoter region might be important for the tissue and cell type specific expression of parvalbumin.

CMB 41

DNA POLYMERASE δ, A NOVEL ENZYME INVOLVED IN DNA SYNTHESIS

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DNA polymerase (pol) δ , a recently described enzyme, has specific properties suggesting an important role in DNA replication. Its associated 3'-5' exonuclease activity, its proliferating cell nuclear antigen/cyclin regulated processivity and the absence of an associated primase make pol δ a promising candidate for leading strand synthesis. In order to clarify the possible relationship of polδ to polα , the latter being a well known replicative enzyme, we have established an assay, based on specific inhibitors and monoclonal antibodies that permits the accurate determination of pol δ and pol α activities in crude extracts. Our results indicated that under very different extraction conditions polδ and polα can be obtained in equal activity and support the idea that pol δ and polα might replicate in a dimeric complex in which polδ could act as the leading and polα as the lagging strand replicase, respectively. Dates showing biochemical and functional properties of polδ will be presented.

CMB 42

A COMPLEX DNA INVERSION SYSTEM OF THE P1 PROPHAGE-RELATED PLASMID

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The phage P1-related plasmid p15B of E. coli 15T$^-$ carries within its 94 kb-long DNA a complex inversion system. It is related to a family of prokaryotic DNA inversion systems found in bacterial chromosomes and phage genomes. This p15B inversion system carries within a 4.7 kb DNA segment a gene for a site-specific recombinase, a cis-acting recombinational enhancer element and six crossover sites. DNA inversion at these sites can yield 240 isomeric configurations of the plasmid. The DNA sequence of an 8.3 kb p15B fragment that carries the inversion system has been determined and analysed. The results suggest that inversion regulates the expression of a protein that has a constant carboxyl-terminal part and a variable amino-terminal part. Its function is not yet known. The comparison of the DNA- and predicted aa-sequences with the corresponding sequences of the other inversion systems surprisingly revealed that the p15B system is more closely related to the inversion system found in the chromosome of E. coli than to the one of the related prophage P1.

CMB 43

TOC: A TRANSPOSON WITH AN UNUSUAL LTR ARRANGEMENT

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..The 5.7 kb TOC (transposon of *Chlamydomonas*) element was isolated by cloning a mutant photosynthetic gene (OEE1) from the strain FUD44. Insertion of TOC into intron 2 of the OEE1 gene in FUD44 prevents OEE1 mRNA accumulation. Three features of TOC are unusual: First, there is no target site duplication at the point of TOC insertion at the OEE1 locus. Second, the left end of the element appears to only contain a fraction of the long terminal direct repeat (LTR) the remainder of which is found immediately adjacent to and internal to the right LTR. Third the terminal bases of the element do not conform to the 5'TG..CA 3' consensus of viral-like retrotransposons. If TOC is a retrotransposon it may be expected to encode a reverse transcriptase and give rise to a near full length transcript. An oligonucleotide (14mer) corresponding to a conserved stretch of reverse transcriptase hybridizes strongly to an internal region of TOC. A full-length TOC transcripts is indeed found in wild type cells but unexpectedly a larger transcript is present in FUD44 cells. Two classes of FUD44 revertants that can evolve O_2 arise from incomplete excision of TOC which leaves behind a complete or fraction of a solo LTR. Revertants FUD44R2 and R3 can accumulate OEE1mRNA but not to wild-type levels. The copy number of TOC elements varies widely in different *C.reinhardtii* strains (10-40) and is much reduced in species interfertile with *C.reinhardtii*. Tetrad analyses show that TOC elements are not closely linked, consistent with the view that these elements are scattered over a number of chromosomes.

CMB 44

Determination of UV-induced DNA-repair in patients with skin neoplasms using a thymine dimer specific antibody

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The initial phase of UV-induced DNA-repair was analysed with a monoclonal antibody highly specific to thymine dimers. To determine the UV-induced DNA-damage and the kinetics of its repair the binding capacity of the monoclonal antibody was analysed by an ELISA. Cultivated fibroblasts of patients and controls were irradiated with UV-light (254 nm; 10 J/m^2) and allowed to recover for varying periods (0. 10. 30. 60, 90 and 120 minutes). The binding capacity was calculated as antibody bound per ng DNA and the "0-minutes"probe was taken as 100% of inducible damage.

The results listed were achieved using cell cultures from patients with the following diseases: basalioma: no differences as compared to healthy controls; malignant melanoma: individual kinetics possibly due to subgroups: familial, multiple and/or sporadic melanoma; dysplastic nevus syndrome: delayed decrease of antibody binding during the first 90 minutes after UV-irradiation.

CMB 45

THE HIN RECOMBINATIONAL ENHANCER ACTIVITY DOES NOT DEPEND ON HOST FACTOR PROTEIN (FIS) BINDING ALONE

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We constructed a series of Hin enhancer mutants. These included between 2-bp and 12-bp insertions and a 2-bp deletion at the ClaI site of the enhancer sequence. Only two of the 10-bp insertion mutants had preserved the enhancer activity. In vitro binding studies of the host factor protein FIS(Factor for Inversion Stimulation) were carried out with the wild type enhancer and its mutants. FIS stimulates the in vitro inversion reaction severalfold and it binds specifically to the enhancer sequence. Using a gel retardation assay we see an increasing number of DNA-protein complexes with increasing amount of FIS protein, irrespective whether the mutant enhancer sequence is active or not. We also examined the actual binding sites of FIS to the mutant enhancer sequences by DNAseI protection experiments. All mutants derivatives studied revealed the same 2 binding sites as the wild type enhancer sequence and they are protected against DNAseI cleavage at the same FIS protein concentration. The mutation sites are between the 2 protected binding regions.

CMB 46

A FLOW CYTOMETRIC METHOD FOR THE DETECTION OF DNA-STRAND BREAKS IN SINGLE CELLS

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Effects if irradiation and bleomycine, which induced DNA-strand breaks, were studied in single cells using a modification of the alkaline unwinding method to quantify DNA-strand breaks. In order to get the damage-dependent ratio of single-stranded to double-stranded DNA, cells were stained with acridine orange. Flow cytometric analyses of green fluorescence (double-stranded DNA) to red fluorescence (single-stranded DNA) were used as a measure of DNA damage (Rydberg, 1984, Int. J. Radiat. Bio., 46, 521).

Primary damage in single cells (M3-1) irradiated with X-rays show no cell cycle stage dependence at the resolution achieved so far. Irradiated cells, which were incubated at 37°C to allow DNA-strand break repair, show a time dependent increase in the double-stranded fraction amount of DNA and reach control values in 30 minutes. Primary damage and repair kinetics with X-rays are compared with the other DNA-strand break inducing agents.

CMB 47

PLASMID ASSAY FOR THE REARRANGEMENT OF THE IMMUNOGLOBULIN HEAVY CHAIN GENE

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Immunoglobulins are encoded by genes which are assembled from V, D and J segments in an ordered series of somatic DNA recombination events during lymphocyte differentiation. These genome rearrangements are probably catalyzed by a specific enzyme or enzyme complex, called DNA recombinase. In order to search for such an enzyme activity, we established a plasmid assay with part of the nonrearranged mouse immunoglobulin heavy chain gene cloned into a pUC18 vector. The HindIII-KpnI insert contains the diversity segment D_{Q52} and the joining segment J_{H1} in a pre- rearranged state. With this plasmid assay we analysed various crude extracts from pre B cells (38B9) and monitored fractions of extracts.

CMB 48

CLONING A MALARIAL GENE FOR AN ENZYME OF THE PYRIMIDINE PATHWAY BY COMPLEMENTATION IN YEAST

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The orotidine 5'-monophosphate decarboxylase of P.falciparum has been cloned by complementation in a yeast ura3,leu2-auxotroph from a genomic library in the shuttle vector YEp13 (which carries LEU2). The gene product is of considerable interest as a possible target for anti-malarial drugs because of differences from the mammalian enzyme. Although there are significant rearrangements in some of the plasmids reisolated from the transformants, several stable plasmids which still retain all of the known properties of YEp13 were established. Further analysis of one of these will be presented. In this plasmid the insert is ~10kb and complementation is also possible, albeit it with a lower efficiency, in an E.coli strain deficient in the same enzyme (pyrF). Promoter usage, gene size, and comparison with homologous genes from other species will be discussed.

CMB 49

DIRECT DETECTION OF THE VIRAL GENOME IN LYMPHOCYTES OF HIV-INFECTED INDIVIDUALS AS A NEW DIAGNOSTIC PROCEDURE

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There is growing evidence that certain individuals infected with HIV virus can remain seronegative for several months and even years. This causes a real concern, as serological assays are the only available routine procedure. In addition, children born from seronegative mothers cannot be tested by serology. There is thefore a pressing need for a direct test of the presence of HIV virus and unfortunately hybridization experiments (Southern blots) are negative on DNA from PBL because of the small percentage of infected cells. We have used oligonucleotides specific for the gag protein of HIV 1 to amplify segment of viral DNA with DNA polymerase several hundred fold and have then hybridized the enzymatically amplified DNA with radioactive cloned HIV 1 DNA. In all cases tested, which included seropositive asymptomatic individuals and AIDS patients, we can detect a strong hybridization signal. This allows for the first time to directly identify the HIV genome in infected individuals.

CMB 50

BACTERIAL SYNTHESIS OF A ECHINOCOCCUS MULTILOCULARIS SPECIFIC ANTIGEN WITH A POTENTIAL VALUE FOR IMMUNODIAGNOSIS.

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The larval stage (metacestode) of the fox tapeworm E. multilocularis causes alveolar echinococcosis in man and some animals (mainly rodents), which serve as intermediate hosts for tapeworm development. One of us (BG) has isolated a species specific antigen (Em 2a) which has already been used as diagnostic tool for seroepidemiological studies in hyperendemic (Alaska) and endemic regions (Switzerland). Since the Em 2a antigen cannot be isolated biochemically from E. multilocularis metacestode tissue in sufficient amounts for carrying out more extensive studies, we intend to produce an appropriate antigen in E. coli. Several antigen producing clones from an E. multilocularis cDNA expression library have been isolated so far. One of these clones expresses a protein which shows a specific immunoreactivity to about 90 per cent of sera from patients with alveolar echinococcosis. Subcloning of the corresponding DNA sequence into plasmid expression vectors led to increased antigen production in bacterial cultures. Purification and serological testing of this recombinant antigen are currently in progress.

CMB 51

RETROVIRUS-MEDIATED TRANSFER AND EXPRESSION OF DRUG RESISTANCE GENES IN MURINE HEMATOPOIETIC PROGENITOR CELLS

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Gene transfer into pluripotent hematopoietic stem cells has been accomplished using retroviruses, however expression of introduced sequences has been low and was progressively shut off in hematologically reconstituted animals. To improve in vivo expression in pluripotent hematopoietic stem cells, we have constructed retroviral vectors containing the neomycin (NEO) resistance gene under the control of the retrovirus LTR and a mutant DHFR gene for methotrexate (MTX) resistance under the control of a variety of promoters placed internally in the retrovirus. Primary hematopoietic cells were infected with recombinant virus and transplanted into lethally irradiated mice. Vectors using hCMV as an internal promoter conferred both NEO and MTX resistance to hematopoietic cells obtained from transduced spleen colonies . Analysis of RNA from individual spleen colonies confirmed the presence of both the LTR and the hCMV driven transcripts .

CMB 52

MOLECULAR BASIS OF THE Mx GENE DEFECTS IN BALB/c AND CBA/J MICE

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The interferon-regulated Mx gene of the mouse encodes the 72 kD nuclear Mx protein that selectively inhibits the replication of influenza virus. Mice carrying Mx+ alleles synthesize Mx protein and resist influenza virus infection. Mice homozygous for Mx- alleles fail to synthesize Mx protein and, as a consequence, are influenza virus-susceptible. Genomic Southern blot analysis allowed to group "old" inbred mouse strains into three discrete RFLP classes: (i) the Mx+ strains A2G and SL/NiA, (ii) BALB/c and 33 other Mx- strains and (iii) CBA/J and 2 other Mx- strains. Mx cDNAs of A2G, BALB/c and CBA mice were cloned and sequenced. Compared to Mx mRNA of A2G, Mx mRNA of BALB/c mice has a 424 nt deletion in the coding region (position 1119 to 1543) resulting in a frame shift and premature termination of Mx protein synthesis. The missing sequences correspond exactly to Mx gene exons 9 through 11. These three exons together with some flanking intron sequences are deleted from the BALB/c genome. Mx mRNA of CBA mice has a point mutation at position 1378 that converts the lysine codon AAA into the stop codon TAA, thus leading to the synthesis of a truncated Mx protein. Analysis of a panel of "new" inbred strains which were directly derived from wild mice showed that all 3 RFLP classes of old inbred mice are present in the wild.

CMB 53

TUBULIN GENES IN TRYPANOSOMA BRUCEI

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Tubulin genes of T. brucei are clustered in an array of alternating α- and β- genes, containing 10 β/α tandem repeats. Outside of the cluster no single copy genes are found. The coding regions are separated by 634 (β/α) and 333 (α/β) bp. Our sequencing data show that the 5' end of the cluster starts with about 95% of an α/β intergenic region, followed by a whole β-gene. At the 3' border, the cluster ends with a truncated gene (containing 68% of β-coding sequence).This particularity has not been found in other clusters so far. Both α- and β-mRNAs are polyadenylated and carry a 39 nt mini-exon sequence at their 5' ends. There is now strong evidence that the mini-exon is added by a trans splicing mechanism. By means of nascent RNA we have shown that the intergenic regions are transcribed at the same rate as are the genes themselves, whereas no stable transcripts thereof have been detected. These results suggest that the tubulin cluster is transcribed in a polycistronic messenger which is rapidly processed into the mature mRNAs.

CMB 54

EXPRESSION IN VITRO OF HEPATITIS A VIRUS REPLICATIVE FUNCTIONS

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We have predicted by computer analysis the organization of the Hepatitis A Virus (HAV) RNA genome. HAV RNA is putatively translated to a polyprotein of genomic size. The P3 region of this polyprotein should contain contiguously four proteins designated 3A, VPg (Virus Protein genomelinked), 3Cpro (a protease that processes the polyprotein), and 3Dpol (RNA polymerase). To test our hypotheses, P3 was expressed in two types of vectors: i) T7ES a plasmid for transcription and translation in vitro ii) vT7 a vacciniavirus recombinant expressing P3 under control of a T7 promoter in presence of a second vacciniavirus recombinant expressing T7 polymerase in eukaryotic cells. Analysis of products with rabbit antibodies raised against synthetic peptides shows that both expression systems generate P3 peptide and products of P3 autocatalytic processing. In vT7 the precursor P3 has the size expected, whereas the T7ES product is truncated within 3Dpol. Our results demonstrate the presence of 3A, VPg and 3Cpro in HAV.

CMB 55

ORGANIZATION OF THE MURINE MX GENE AND CHARACTERIZATION OF ITS INTERFERON-INDUCIBLE PROMOTER

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Specific resistance of mice to influenza virus is due to an IFN-induced protein designated Mx. The murine Mx gene consists of 14 exons, distributed over at least 55 kb of DNA. The Mx promoter and the first exon, comprising a non-coding sequence of 29 bp, are separated by an intron of >30 kb from the second exon, which contains the first codon of the coding sequence. A cluster of transcription initiation sites is preceded by a TATA box and an Sp1 binding site. The 5′ boundary of the promoter region required for maximal induction by IFN is about 140 bp upstream of the initiation sites; at least 2 distinct regions contribute to the IFN response. A consensus sequence of the type PuGAAAN$_{1-2}$GAAAPyPy is present in all IFN-inducible promoters. A 32-nucleotide segment of the Mx promoter containing this sequence, or a tetrameric repeat of GAAACT, which occurs in the Mx promoter, placed between the SV40 enhancer and the TATA box of the ß-globin promoter mediated the IFN response. The Friedman-Stark consensus sequence is not represented in the Mx promoter.

CMB 56

IDENTIFICATION OF THE EXTRA SEX COMBS GENE BY SEQUENCE ANALYSIS OF THREE ESC MUTANT ALLELES

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The extra sex combs (esc) gene of Drosophila shows remarkable developmental and regulatory characteristics: (i) it restricts the spatial expression of homeotic genes, particularly those of the bithorax- and Antennapedia-complex, (ii) it is itself homeotic, and (iii) in contrast to most other homeotic genes, it exhibits a maternal effect. Previously, we have tentatively identified the esc gene with the only transcription unit detected within a 12 kb genomic DNA fragment used successfully for P-element mediated gene transfer to rescue the esc phenotype. We have now corroborated this supposition by sequencing three esc mutant alleles, esc^1, esc^2, and esc^6. The three mutant alleles contain nucleotide changes that alter the esc protein in different ways. The esc^6 mutation produces an altered splice donor site, the esc^2 allele exhibits a frameshift mutation, whereas the esc^1 allele has two altered amino acids. To analyze the location and spatial distribution of the esc protein, we are currently raising antisera against it.

CMB 57

SEQUENCES RESPONSIBLE FOR REGULATION OF THE PIG UROKINASE-TYPE PLASMINOGEN ACTIVATOR GENE BY TPA AND cAMP.

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The urokinase-type plasminogen activator (uPA) gene is positively regulated transcriptionally by both the tumor promoting phorbol ester, TPA, and by cAMP in the pig kidney epithelial cell line LLC-PK$_1$. Using stable transfection of LLC-PK$_1$ cells with uPA gene constructs, show that the sequences responsible for regulation by TPA and by cAMP, and for basal uPA gene expression reside within 4 kbs of the 5′ flanking region. We used Dnase I protection techniques to examine the specific DNA-protein interactions responsible for induction. We plan to characterize further the regulatory proteins by biochemical and molecular biology approaches.

CMB 58

THE GENE NETWORK CONCEPT

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Based on evolutionary considerations, we have proposed that genes controlling complex functions such as segmentation, DNA replication, and metabolic pathways are integrated into networks by the principle of independent assortment of a limited number of different domains, each defining a homologous set. The diversity of genes belonging to a network would be generated by two variables, namely first by alterations in the sequence of a particular homology and second by the many different ways in which different domains may be combined with each other. For example, genes that determine segmentation in Drosophila embryogenesis are integrated into a network by sharing a relatively small number of characteristic domains. If our concept is correct, it should be possible to isolate the genes belonging to a particular network on the basis of structurally homologous domains by starting from a single isolated gene which is part of the network. A scan of the segmentation gene paired for homologous domains shared with other genes of Drosophila has provided preliminary support for this concept.

CMB 59

ISOLATION AND EXPRESSION OF cDNA CLONES ENCODING HUMAN MX AND AN MX-RELATED PROTEIN

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The murine Mx gene is induced by type I IFN and confers selective resistance to influenza virus. Mx protein in murine cells is detected exclusively in the nucleus while human Mx-protein is in the cytoplasm. We have isolated two different, Mx-related cDNAs from IFN-induced human T98.G cells. We determined by sequence and Southern analyses that there are two different Mx-related genes in man, designated C and E, both related to the murine Mx gene. Because the human Mx-C gene is more similar to the murine Mx gene than to the human Mx-E gene we conclude that duplication of the Mx genes occurred prior to mammalian radiation. Therefore, most mammalians will likely contain two or more Mx genes. Both human Mx genes are induced upon IFN-α treatment in several cell lines tested, however at different levels and in different ratios. In HeLa cells the human Mx-E gene is expressed after IFN-induction, whereas the Mx-C gene is not. Transient, constitutive expression of the human Mx-C cDNA in cos cells led to protection of these cells against influenza virus replication.

CMB 60

REGULATION OF EXPRESSION OF THE cAMP-DEPENDENT PROTEIN KINASE GENES

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Several isotypes of the regulatory (R) subunit of the cAMP protein kinase (PKA) have been identified. cDNA clones coding for 3 different R subunit proteins, RI, RII52 and RII54 have been isolated. Northern blot analysis using specific probes showed that RI was expressed in all the pig tissues analyzed whereas the expression of RII54 was high in muscle and heart, and RII52 was only detected in brain and ovaries. In order to gain a better understanding of the regulation and tissue specific expression of the various PKA regulatory subunits, cloning and characterization of the corresponding genes was started. The gene coding for RI has been isolated and the nucleotide sequence of the promoter region determined. It contains several motifs characteristic of house-keeping genes; in addition, an 8 bp sequence (TGACGTCA) was identified which corresponds to the core sequence of the cAMP responsive element (CRE), described for several cAMP induced genes. Footprint analysis using LLC-PK1 cell extracts show that the region containing the CRE was protected. Work is in progress to determine the possible functional involvement of this element in RI transcription regulation following cAMP elevation. Recent data suggest that, in LLC-PK1 cells, cAMP elevation following hormonal stimulation leads to an increase in RI mRNA levels.

CMB 61

DIRECT CLONING OF 5' REGULATORY SEQUENCES AND SELECTION OF COORDINATELY CONTROLLED PROMOTERS

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We have characterized and purified (Mueller and Schibler) a transcription factor involved in liver-specific expression of the albumin gene. Evidence indicates this protein is EBP/CBP. To test whether it has a more general role in liver-specific gene expression, we searched for binding sites in other liver-specific promoters. For this we designed a protocol allowing direct screening of genomic libraries for promoter sequences highly active in hepatocytes. As hybridization probes, we used short fragments of poly-A^+ RNA chemically decapped and enzymatically recapped with α-P GTP. Hybridizing clones were assumed to contain the 5' end of the gene. This was confirmed by S1 analysis of selected clones. Several clones were highly liver specific, while others were members of the α2u-globulin gene family. To test these clone for EBP/CBP binding sites, we performed a filter-binding assay using heat-treated liver nuclear extracts. The α2u-globulin clones and another, as yet identified, clone contained restriction fragments which were specifically retained Footprint and competition analysis with specific oligonucleotides confirmed the presence of binding sites, suggesting possible coordinate regulation in hepatocytes.

CMB 62

ONE B-CK GENE GIVES RISE TO TWO B-CK ISOPROTEINS

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Brain-type creatine kinase (B-CK) is expressed at high levels in brain, smooth muscle, chicken heart and in many embryonic tissues. Purified B-CK subunits from several sources can be dissociated into two major species on 2D-gels; a basic B-CK and an acidic B-CK isoprotein. The basic species comigrates with the protein produced from cell free translation of RNA transcribed "in vitro" from the full length B-CK cDNA clone H4. Analysis of other clones have revealed the existence of 3 types of B-CK like sequences: 18c, 18b and 18e in addition to H4. The 18c DNA diverges from the H4 sequence at the amino terminal segment and the resulting amino acid composition might be identical to the acidic B-CK isoprotein.

Comparisons of cDNA sequences H4 and 18c with partial genomic B-CK sequences show that these two transcripts are encoded by one B-CK gene. The distribution of the coding sequences within the gene strongly suggest that the two mature transcripts arise by differential splicing.

CMB 63

A VIRAL RNA POLYMERASE CAUSING BIASED ERRORS IN A PERSISTENT INFECTION OF THE HUMAN BRAIN

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RNA virus replication infidelity results in rapid genomic evolution, allowing adaptation to altered selective pressures. In persistent RNA virus infections lack of selection for fully competent virus correlates with accumulation of genomic mutations. Until now it was unclear whether accumulation of mutations could be increased by particularly imprecise viral RNA polymerases. We are studying persistent infections of measles virus (MV) in human brains, causing the rare but always lethal syndromes subacute sclerosing panencephalitis (SSPE) and measles inclusion body encephalitis (MIBE). We have cloned full length copies of MV mRNAs from brain autopsies of two SSPE and one MIBE patients. From these cDNAs synthetic mRNAs were produced and translated *in vitro*. Whereas in the two SSPE cases generally only small deviations from the standard pattern of protein production were monitored, in the MIBE case the expression of the dispensable viral envelope proteins was drastically altered or cancelled. Surprisingly we found that in all copies of the MIBE matrix mRNA analysed about 50% of the U (as read in the plus strand) mutated to C. The other nucleotides mutated in a random fashion at a 20 times lower level. This effect, monitored in the MIBE but not in SSPE cases, must be due to an altered MV polymerase, introducing preferentially one kind of transitions in one strand of the MV genome (U to C in plus strand or A to G in minus strand). The nucleocapsid gene did not show this bias, indicating that in this non-dispensable gene mutations are fixed at a much lower rate. To our knowledge this is the first direct evidence for a polymerase introducing biased errors.

CMB 64

DEFECTIVE PARTICLES IN CELL CULTURES INFECTED WITH HEPATITIS A VIRUS

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In contrast to the majority of the picornaviruses, Hepatitis A virus (HAV) leads to persistent rather than to lytic infection in most cell culture systems. This virus/cell relationship may be brought about by the presence of defective interfering particles which contain incomplete, deleted genomes. Particles with the respective physical characteristics were readily found in harvests of various HAV strains grown in different cell lines, at low as well as at high passage level. Three major deletions mapped in the region of the genome coding for structural proteins. Beside, a series of truncations could be located near the 3'end of HAV-RNA. Under one-step growth conditions defective and standard genomes appear in virus particles simultaneously. No interfering activity of the defective particles could so far be established.

CMB 65

MAPPING OF A MINOR TRANSCRIPT PRESUMABLY CODING FOR STRUCTURAL PROTEIN VP1 OF AAV (ADENO-ASSOCIATED-VIRUS)

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The single-stranded DNA genome of AAV is 4674 nucleotides long and contains 3 promoters at 5, 19 and 40 map units. The P40 promoter regulates the expression of the capsid gene which codes for the structural proteins VP1, VP2 and VP3. From the 2.6 kb primary transcript nucleotides 1907 to 2227, located in the non-translated 5' region of the transcript, are removed to yield a 2.3 kb mRNA. From this spliced mRNA the 2 smaller capsid proteins VP2 and VP3 are synthetized by initiation at differential start codons. The exact coding region for the largest capsid protein VP1, however, is not known. Using the published AAV sequence generation of VP1 -in accordance to other parvoviruses- could be best explained by the occurence of an additional mRNA spliced from the 2.6 kb primary transcript. To detect this putative mRNA we have used S1-mapping as well as direct mRNA sequencing with synthetic primers. A minor acceptor site was found at nt 2200. We have also sequenced the relevant regions on the DNA level in order to confirm the published sequence.

CMB 66

ANALYSIS OF *Dictyostelium discoideum* GENES RELATED TO MAMMALIAN *ras* AND *myc*

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The already characterized *Dictyostelium ras* gene (Dd *ras*) generates two transcripts during development. We mapped the 5' end of the mRNA by primer extension, but cannot yet fully understand the size difference between the two transcripts. Further mapping of the mRNAs is in process.

To investigate the presence of other *ras* related genes in *Dictyostelium*, we used a synthetic oligonucleotide devised from the conserved sequences of *ras* proteins from different organisms. We can detect at least 5 independent sequences on genomic DNA blots, one of which is the *ras* gene itself. We cloned and sequenced a cDNA corresponding to a second of these sequences. The encoded protein, however, originate from the opposite strand and does not show significant homology to *ras*. The other crosshybridizing sequences are currently under investigation.

Using the same approach, we can detect one genomic sequence which hybridizes to two oligonucleotides derived from exon 2 and 3 sequences conserved between mammalian and avian *myc* genes. We are currently screening cDNA and genomic libraries with these oligonucleotides.

CMB 67

IMMUNOGLOBULIN VARIABLE REGION GENES IN THE HYBRIDOMA CE7 AND IN VARIANTS EXPRESSING A DIFFERENT ISOTYPE

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The monoclonal antibody CE7 ($IgG_1/\ $) recognizes a 190 kd glycoprotein on the cell surface of neuroblastoma cells. It binds strongly to all neuroblastoma tested so far and only weakly to adrenal medulla. No binding was detected to any other normal or neoplastic tissue. We have isolated variants of CE7 which express antibodies of the isotypes IgG_{2b} and IgG_{2a}. These switch variants occur at a frequency of 10^{-5} and have the same specificity as CE7. We have identified the functionally rearranged variable gene segments by Southern analysis and isolated them from a genomic library in the Phage EMBL4. We are currently constructing chimeric light and heavy chain genes with these mouse sequences and human constant region gene segments.

CMB 68

EPITOPE MAPPING OF HLA-DR ANTIGENS WITH THE USE OF DNA-TRANSFECTED CELLS. C Berte, J Gorski, W Reith and B Mach, Department of Microbiology, University of Geneva, .

This study reports on a direct correlation between individual polymorphic markers (serological determinants) and the products of individual class II genes and alleles. The DR product of loci DR B1 and DR B3 (alleles 52a, 52b and 52c) from three DRw6 and two DR3 haplotypes, all with known DNA sequences, have been expressed in DNA-transfected Ia negative mouse cells. Four major patterns of reactivity of anti-DRw52 anti-sera and monoclonal antibodies (MAbs) with these transfectant lines have been identified :i) the product of both the 52a allele of locus DR B3 (certain DR3 and DRw6 haplotypes) and the DR B1 locus of DR3 react with certain MAbs and antisera (TR81). This epitope is the phenotypic counterpart of the gene conversion event which generated the DR3 gene. ii) the product of the 52b allele of locus DR B3 reacts with specific antisera (TR22). iii) certain anti-DRw52 MAbs react with the 52a and 52b alleles of DR B3 as well as with DR B1 of DR3. iiii) other anti-DRw52 McAbs react with the gene products of both DR B1 and B3 of DR3 and DRw6. This study has allowed us to map specific DRw52-like epitopes to individual genes and to individual amino acids in the sequence of the various DR B chains.

CMB 69

CHROMATIN FOLDING MODULATES NUCLEOSOME POSITINING IN YEAST MINICHROMOSOMES

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Due to the protection of DNA by histone proteins, the position of nucleosomes with respect to the underlaying DNA sequence might play a decisive role in regulation of gene expression and replication. Examples where histone-DNA interactions and flanking nuclease sensitive regions determine nucleosome positions have been reported (Thoma and Simpson, 1985, Nature, 315, 250; Thoma, 1986, JMB 190, 177). Here, two circular minichromosomes were constructed which were designed to form one tight tetranucleosome each. The tetranucleosome was found in the long plasmid only, while the short plasmid adopted an alternative structure. The tetranucleosome seems to be a structure too stiff to be accommodated in the small plasmid. The nucleosome positions, therefore, appear to be modulated by the three dimensional folding of chromatin. Furthermore, the structures show clearly that histone-DNA interactions are not sufficient determinants of nucleosome positions.

CMB 70

CHROMATIN STRUCTURE OF YEAST GENES: DNA MEDIATED EXCLUSION OF NUCLEOSOMES AT 3'ENDS AND CONSTITUITIVE PROMOTERS?

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Due to the protection of DNA by histone proteins, the positions of nucleosomes relative to the DNA sequence might play an important role in gene regulation and expression. A poly (dA-dT) sequence is an upstream element for constituitive expression of the DED1 gene in S. cerevisiae (Struhl, K., 1985, PNAS 82, 8419). It is closely linked to the 3'end of the HIS3 gene (Struhl, K., 1985, NAR 13, 8587). We have subcloned these sequences into yeast minichromosomes and analysed their chromatin structures. In all constructs, the HIS3 3' end and the DED1 promoter, including the poly (dA-dT), reside in a nuclease sensitive region flanked by positioned nucleosomes. In analogy to the structure of the 3'end of the URA3 gene, part of the nuclease sensitivity is related to the end of the gene. The other half might be due to the poly (dT-dA) sequence (Struhl, o.c.). In vitro reconstitution experiments are in progress to investigate if the DNA sequence alone excludes nucleosome formation or if additional factors are required.

CMB 71

PROTEIN-DNA CROSSLINKING IN SOLUBLE RAT LIVER CHROMATIN USING AN UV PULSE LASER

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We have established the crosslinking of histones to DNA in soluble rat liver chromatin using a 248nm-15nsec pulse laser.
The following experimental approaches have been used to test for UV-induced histone-DNA crosslinking:
1) In agarose gel electrophoresis under dissociating conditions, irradiated nucleosome cores give rise to a band shift in 20-30% of the ethidium bromide stained material.
2) 12-15% of the DNA of irradiated core particles are trapped in a nitrocellulose filter binding assay.
3) Up to 50% of the DNA of irradiated rat liver chromatin coprecipitate with proteins. The radical-scavenger beta-mercaptoethanol reduces the crosslinking efficiency by about 10%.
4) For the identification of the crosslinked histones, the protein components of isolated complexes were analyzed by SDS-PAGE. Mainly H3 was found to be bound, followed by H2B, H2A and H4 in decreasing order.
In all four approaches, the crosslinking efficiency was on the order of 1.5-3% at 100-200mJ. The method is now applied to the ribosomal genes of Dictyostelium discoideum to elucidate the arrangement of proteins in different gene regions and transcriptional states.

CMB 72

CORRELATION BETWEEN GENE ACTIVITY AND CHROMATIN STRUCTURE IN RIBOSOMAL GENES OF FRIEND CELLS.

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The chromatin structure along the ribosomal genes of Friend erytroleukemia cells was analyzed under conditions of different transcriptional activities: logarithmically growing cells for high transcriptional activity, stationary cells for low transcriptional activity, and metaphase cells for inactive ribosomal genes. Micrococcal digests as well as the mobility of psoralen-crosslinked restriction fragments from the coding region indicate that in interphase cells the ribosomal genes are organized into two different populations (possibly active and inactive gene copies). No difference was seen between the chromatin structure of interphase nuclei from stationary and from logarithmically growing cells. This suggests that the overall active chromatin organization is independent from the transcription process. However, a clearly nucleosomal structure was found in metaphase chromosomes.

CMB 73

SEQUENCE SPECIFIC PROTEIN-DNA INTERACTIONS IN THE DISTAL PROMOTER REGION OF A CELL CYCLE REGULATED HUMAN HISTONE GENE

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Western blots of electrophoretically fractionated nuclear proteins were hybridized with a sequential series of 5' endlabeled DNA segments representing 1070 nucleotides 5' and 800 nucleotides 3' to the mRNA coding region of a H4 histone gene. Specificity of binding was established by eluting the radiolabeled DNA fragments associated with the various protein bands and then electrophoresing the eluted DNA's in a second dimension. Identification of the proteins was confirmed by repeating the analysis with Western blots of electrophoretically fractionated total histones, histone H1 and residual nuclear proteins. Protein binding was observed in the region located between -615 bp and -1070 bp upstream of the ATG codon and included a histone H1 binding site flanked on both sides by binding sites for a 45 kd nuclear protein. This segment of the gene contains a DNase I sensitive region in the center and sequence analysis revealed the presence of scaffold attachment and topoisomerase II concensus sequences as well as possible regulatory elements for the expression of this gene. Our results represent the first identification of nuclear proteins that preferentially interact with segments of a gene that may mediate association with a nuclear matrix and may be involved in the regulation of gene expression.

CMB 74

CAUSAL RELATION BETWEEN LYME-BORRELIOSIS AND MULTIPLE SCLEROSIS (MS)?

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Lyme-Borreliosis is a recently discovered infection by Borrelia burgdorferi (Bb) causing dermatologic, rheumatologic, cardiac and neurologic disorders. Presently,on the basis of epidemiological studies, it seems questionable to assume a causal link between MS and Lyme-Borreliosis.Nevertheless there are reports on MS-like conditions in Bb sero-positive patients,which resolve after antibiotic treatment. In order to approach this question,we studied the binding of serum and cerebrospinal fluid (CSF) immunoglobulins on blots of B.burgdorferi.About 10% of MS patient samples did show cross-reactivity with Bb antigens.
Results of a current investigation on a possible correlation between the oligoclonal banding pattern of the CSF immunoglobulins of MS cases and their antibody response to Bb antigens will be presented (Supported by a grant from the Swiss MS Society).

CMB 75

DIFFERENTIAL TRANSLATION OF *caudal* mRNA DURING EARLY EMBRYOGENESIS OF *DROSOPHILA* REQUIRES *bicoid* GENE ACTIVITY

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Caudal (cad) is a *Drosophila* gene that is involved in segment specification along the antero-posterior (A/P) axis. It contains a homeobox. Using antibodies directed against the *cad* protein, we have determined the expression and distribution of *cad* in wildtype and mutant *Drosophila* embryos. In wildtype embryos, prior to the cellular blastoderm, *cad* protein is expressed as a gradient along the A/P axis. This assymetric protein distribution appears prior to any detectable differences in mRNA localization suggesting that translational control is involved. We find that in maternal-effect mutants that affect head development the *cad* protein gradient is destroyed or altered. These mutants are either *bicoid (bcd)*, or those that affect the localization of *bcd* activity. Mutants that affect posterior development, namely the abdomen, do not have an effect on the *cad* gradient. These findings suggest that in anterior parts of the embryo the *bcd* product mediates either directly or indirectly the translational repression of *cad* mRNA. This is supported by the fact that the observed changes in *cad* expression are reciprocal to changes in *bcd* localization.

CMB 76

DETECTION OF GLIA-DERIVED NEXIN IN THE OLFACTORY SYSTEM OF THE RAT

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Glia-derived nexin (GdN) is a 43kd protease inhibitor with neurite promoting activity which is isolated from the medium conditioned by C6 rat glioma cells. Specific polyclonal antisera have been raised against it. These antibodies stain a single band at 43kd when the concentrated conditioned medium is analyzed on immunoblots. No crossreactivity with fibronectin, laminin or other constituents of conditioned medium is observed. Immunohistochemistry shows that GdN occurs in the olfactory system of the rat: predominantly in the olfactory nerve layer of the olfactory bulb and in the submucosa. A weaker staining is detected in the olfactory epithelium. Comparative studies with antibodies against fibronectin, glial fibrillary acidic protein and vimentin suggest that GdN antibodies recognize cells which are associated with the olfactory system rather than the olfactory neurons themselves. These results suggest that the presence of GdN in the olfactory system of the rat may be related to the constant degeneration and regeneration phenomena taking place in these structures.

CMB 77

MOLECULAR CLONING OF THE CHICKEN EXTRACELLULAR MATRIX PROTEIN TENASCIN REVEALS HETEROGENEITY AT THE 3'ENDS OF THE cDNAs

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Tenascin, (Tn) is a hexameric, disulfide-linked glycoprotein with highly similar subunits of 190-240 kd. cDNA clones were isolated and characterized by immunological screening of a λgt11 expression library. Antibodies eluted from cDNA-encoded fusion proteins showed the same immunofluorescence staining as the original α-Tn antiserum. On western blots these affinity purified antibodies reacted exclusively with Tn. A monoclonal antibody α-Tn60 reacts with the fusion proteins from the 3'end of the coding region and binds to the central part of the tenascin molecule. Thus the C-terminus of the peptide chains must be in the center where the subunits are joined together. The sequences of the cDNAs showed regions of 100% homology as well as different 3' termini. Northern blot analyses revealed that all three cDNAs hybridized at 10kb. The cDNAs were used to study the regulation of tenascin mRNA expression.

CMB 78

EXPRESSION OF GLIA-DERIVED PROTEASE NEXIN I IN MAMMALIAN BRAIN AND ITS INDUCTION IN TISSUE CULTURE SYSTEMS.

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Glia-derived protease nexin I (GdN) is a 43 kD serine protease inhibitor with neurite promoting activity in neuroblastoma cells. We have shown that GdN shares sequence homology to antithrombin III, and plasminogen activator inhibitor I and II, and therefore belongs to the serpin superfamily. In contrast to other members of this family, GdN is expressed at low levels in the liver, but steady state levels of its transcript are relatively high in different brain regions. In the olfactory system, which has a unique ability for neuronal regeneration in the CNS, both GdN transcript and the gene product can be detected. Sciatic and optic nerve cultures have been used as models of the peripheral and central nervous systems to study the induction of GdN. The results are compatible with the involvement of GdN in regeneration phenomena.

CMB 79

TENASCIN ANTAGONIZES FIBRONECTIN ACTION

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The extracellular matrix is of fundamental importance in directing development and cell differentiation by transmitting signals through membrane receptors influencing cell shape and gene expression. Therefore the effect of two extracellular matrix proteins, tenascin (Tn) and fibronectin (Fn), on cell attachment and differentiation was investigated. Primary chick embryo fibroblasts attach to a Tn substrate, but remain rounded and do not spread out. Tn inhibits integrin-mediated cell attachment to Fn, laminin as well as the GRGDS-peptide. A monoclonal anti-Tn antibody neutralizes the inhibitory activity of Tn and therefore is assumed to bind to the cell-binding site of Tn. On electron micrographs, showing this monoclonal antibody bound to Tn, these epitopes can be localized at the terminal knobs of the six Tn subunits.

CMB 80

EFFECTS OF PALYOXIN (PTX), 12-O-TETRADECANOYLPHORBOL-13-ACETATE (PTA) AND OUABAIN ON GROWTH AND SQUAMOUS DIFFERENTIATION OF HUMAN BRONCHIAL EPITHELIAL (HBE) CELLS IN VITRO.

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The effects of the non-TPA type tumour promotor PTX was studied on HBE cells in an in vitro serum-free system. PTX did not induce squamous differentiation of normal HBE cells. When tested on cells at clonal density, PTX was equally cytotoxic for normal HBE cells, a human tumour cell line, and for adeno 12-SV40 hybrid virus infected HBE cells (BEAS-2B cells). The effect of PTX on interleukin 1-ß and c-myc mRNA steady state in BEAS-2B cells was studied : 1pM PTX increased steady state level of both m-RNA at 12 and 18h, respectively. Further, the induction was accompanied by an increase in thymidine uptake. Since PTX binds to (Na/K)-ATPase, the effects of ouabain were compared to the effects of PTX. A ouabin-resistant cell line was as sensitive to PTX as the parent ouabain-sensitive cell line, suggesting a distinct binding site for PTX and ouabain. Ouabain also increased the steady state level of c-myc gene expression, but earlier than PTX, and the ouabain induced c-myc gene expression was accompanied by a drop in thymidine uptake.

CMB 81

HOMEO BOX CONTAINING GENES IN NEMATODES

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The study of homeo box containing genes in nematodes has been approached by using the homeo boxes of the Drosophila genes fushi tarazu (ftz) and Antennapedia (Antp) as hybridization probes. From a genomic library of Ascaris lumbricoides, several positive clones were isolated and one of them was further investigated. Sequence analyses revealed the existence of a 180 bp long Ascaris homeo box (AHB-1), located within a short open reading frame totally 243 bp long. The AHB-1 encodes an amino acid sequence which is 78% homologous to the Drosophila Antp homeo domain. Hybridization of the AHB-1 DNA probe to Southern transfers of total genomic DNA demonstrates that the Ascaris genome contains at least 11 different homeo boxes. Similar Southern blot hybridization experiments with C. elegans or Parascaris equorum indicate that the genome of these nematodes also includes several homeo box sequences. By using the Ascaris sequence AHB-1 as a probe, we succeeded in isolating two different homeo box containing clones from a C. elegans genomic library. This is the first report on the existence of homeo box sequences within the genome of nematodes, worms which have non-segmented body plans.

CMB 82

CONFOCAL MICROSCOPY: OPTICAL SECTIONING IN FLUORESCENCE AND REFLECTED MICROSCOPY

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Conventional microscopy implies that the entire field of view is illuminated and imaged simultaneously. With confocal microscopy, a spatial filter is situated in the reflected light path and configured so that the region viewed at any one time is coincident with the diffraction-limited illumination spot. Thus, only those regions of the sample lying within a narrow depth of focus are now imaged. Regions outside this depth of focus appear black rather that blurred. Spatial resolution is also improved and is greatest when incoherent light is used to produce the image. Comparative viewing can be observed in a great variety of applications.

CMB 83

CNS WHITE MATTER IS A NON-PERMISSIVE SUBSTRATE FOR NEURON ADHESION AND NEURITE GROWTH. T. Savio and M.E. Schwab, Brain Research Institute, Univ. of Zürich, 8029 Zürich, Switzerland. (Sponsor: K.Ballmer).

Cultured oligodendrocytes and CNS myelin have been shown to contain specific membrane proteins which exert a strong inhibitory effect on neurite regeneration in culture. In the present study, NB-2A neuroblastoma cells and rat or chicken embryo ganglion cells were grown on frozen sections of adult rat CNS and PNS. After two days in culture, NB-2A and neuronal cells adhered and neurons produced processes almost exclusively on CNS grey matter and on PNS tissue, while central white matter was almost devoid of cells and processes. NB-2A cell cultivated on trout spinal cord (lower vertebrates show CNS regeneration!) grew equally well on grey and white matter regions. NB-2A cells grown on spinal cord sections of rats in which oligodendrocyte proliferation and myelination had been chemically blocked were present also on white matter regions. These data, therefore, suggest that a crucial factor for the lack of regeneration in the CNS is specifically associated with CNS myelin of higher vertebrates.

CMB 84

DIFFERENTIATION OF NEURAL CREST CELLS IN CLONAL CULTURES

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Neural crest (NC) cells of the Vertebrate embryo are the precursors of autonomic and sensory peripheral ganglia, of pigment cells and of some mesectodermal derivatives. Developmental potentials of quail mesencephalic NC cells taken at the beginning of their migration towards the sites of differentiation were evaluated by cultivating them as single cells on feeder layers of 3T3 cells. Two hundred and forty clones were analyzed after 7 to 10 days of culture. Their size varied from one cell to more than 20 000 cells. Phenotype analysis revealed the existence of pluripotent common progenitors for different kinds of neurons, non-neuronal cells and melanocytes as well as of some committed precursors for neurons only. However, the majority of cells gave rise to several different combinations of cell types between these two extremes. These cloning experiments thus demonstrate a striking heterogeneity of NC cells in terms both of their pluripotency and their proliferation potential.

CMB 85

DISTINCT "DEVELOPMENTAL PROGRAMS" FOR CK ISOENZYMES DURING MUSCLE DIFFERENTIATION

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RNA hybridization experiments and immunoblot analysis with isoprotein specific antibodies showed that accumulation of mRNA of each of the three CK species (M-CK, B-CK and Mi-CK) as well as the proteins follow a "developmental program" characteristic for each tissue during chicken embryogenesis. While the more ubiquitously distributed isoprotein B-CK is expressed in early embryonic stages the muscle specific M-CK is detectable in prospective skeletal muscle of embryos older than 8 days. However, Mi-CK appeared in developing skeletal muscle with a considerable lag. B-CK accumulation in skeletal muscle increases until day 11 of incubation, then it begins to level off, while in heart, expression stays at a high level. M-CK is not expressed in chicken heart whereas it replaces B-CK in adult skeletal muscle. Mi-CK accumulation in heart does not increase significantly until hatching, however, in leg muscle of 19 day old embryos Mi-CK is already present at a level comparable to adult muscle. These results suggest that the expression of the CK genes in the investigated muscle tissues follows different developmental programs.

CMB 86

METALLOPROTEASE-BLOCKERS INHIBIT C_6 GLIOBLASTOMA INVASIVE BEHAVIOUR IN CNS TISSUE. P.A. Paganetti, P. Caroni, and M.E. Schwab (sponsor: J.T. Gasser*) Brain Research Institute, University of Zürich, 8029 Zürich, Switzerland

CNS white matter represents a highly non-permissive substrate for regenerating neurites and migrating cells. Despite this fact the invasive rat C_6 glioblastoma line infiltrates optic nerve explant, spreads well on grey and white matter of cerebellum (frozen sections), and on CNS myelin coated wells. A degrading activity was shown to be present on the surface of C_6 cells, tightly associated with C_6 plasma membranes. Inhibitor studies demonstrated that the metalloprotease blocker 1,10-phenanthroline and the dipeptide Z-YY can specifically inhibit spreading of C_6 cells on CNS white matter, but not on control substrates. Asp-, Ser-, and Cys- protease blockers were totally ineffective. C_6 cells in the presence of the dipeptide Z-YY stopped to spread on white matter but not on grey matter, and their infiltration into optic nerve explants was inhibited. This metalloprotease activity may play an important role in the invasive behaviour of C_6 cells in the CNS.

CMB 87

OLIGODENDROCYTES AND CNS MYELIN CONTAIN INHIBITORY SUBSTRATES FOR NEURITE GROWTH. P. Caroni and M.E. Schwab, Brain Research Institute, Univ. of Zurich, August-Forel-Str.1, CH-8029 Zurich, Switzerland

Cocultures of neurons with explants of optic nerves, with dissociated oligodendrocytes, or on CNS myelin as a substrate all point to a strongly non-permissive substrate component associated with differentiated oligodendrocytes and CNS myelin. Biochemical analysis showed that this property is associated with two major (35kD, 250kD) and a minor protein component (56kD). Addition of these components to good substrates turns these substrates into non-permissive ones, indicating the inhibitory nature of these components. Antibody IN-1 raised against the 250kD component binds to myelin, living oligodendrocytes, and to the three respective bands on western blots. IN-1 strongly reduces the inhibitory properties of myelin and oligodendrocytes. Injection of IN-1 into optic nerve explants led to ingrowth of many axons from cocultured neurons, a result never obtained in non-injected or control antibody injected nerves. These IN-1 antigens may play important roles in CNS development, maintenance and regeneration.

CMB 88

REGULATION OF THE EXPRESSION AND STABILITY OF mRNA FOR INTERLEUKIN 3

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We have found that stability of IL-3 mRNA differs in various cell lines. In leukemia WEHI-3B cells, expressing IL-3 constitutively, the mRNA has a half-life of about 2 hrs. In IL-3 secreting tumor cells derived from PB-3c mastocytes infected with v-Ha-ras, the IL-3 mRNA decays more rapidly (half-life = 30 min.). The fast decay of mRNA can be prevented by the presence of cycloheximide. In PB-3c cells infected with a zip vector carrying IL-3 cDNA (devoid of 3' non-coding AU-rich sequence) the transcribed mRNA is stable. While treatment of cells with TPA did not markedly affect the IL-3 mRNA expression or half-life, the calcium ionophore was found to be a potent stimulator of IL-3 transcription. This suggests a role of the inositol trisphosphate signal transduction pathway in IL-3 gene expression.

CMB 89

SECRETION OF AN HEMATOPOIETIC GROWTH FACTOR UNDER THE CONTROL OF AN INDUCIBLE H-RAS ONCOGENE

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We have introduced by electroporation the human H-ras oncogene under the control of an inducible promoter (Jaggi et al., EMBO J. 5, 2609, 1986) into interleukin-3 (IL-3)-dependent mouse PB-3c mastocytes. We found a correlation between inducible ras-p21 expression and a decrease in the IL-3 requirement. When IL-3 was removed, cells grew without the growth factor provided dexamethasone was present. Supernatants from such cultures secreted a mitogenic factor, presumably IL-3. Factor independent growth continued apparently indefinitely. Removal of dexamethasone led to cell death suggesting that production of the factor is under the control of the inducible ras gene. Analysis of IL-3 transcription in response to dexamethasone is in progress.

CMB 90

ANALYSING THE MECHANISM OF AUTOKRINE IL-3 SECRETION BY CELL FUSION

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We found that introducing v-H-ras into IL-3-dependent, non-tumorigenic mouse mastocytes (PB-3) led to the generation of autokrine IL-3 secreting tumor. To study the mechanism of this autokrine tumoe formation, we performed cell fusion between secreting tumors and the IL-3-dependent parental cell. In 8/8 hybrids, IL-3 production was sub- and tumor formation delayed. Hybrid-derived tumors secreted again IL-3. For control, a fusion between PB-3 and PB-3 cellsinfected with a zip-vector carrying an IL-3 gene (W. Ostertag) was performed. Here, as expected, factor production was dominant. We suggest a recessive mechanism in generating autokrine tumors by v-H-ras.

CMB 91

IN VITRO GROWTH IN SEMISOLID MEDIUM OF STROMAL CELLS FROM HUMAN CARCINOMAS AND NON-TUMORAL EFFUSIONS.

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Human tumour cells can grow as colonies (clonogenic growth) in a semisolid medium. Are stromal cells from the same tumours also able of clonogenic growth ?
The stromal cells (fibroblasts, macrophages) from 25 primary and metastatic carcinomas were cultured in methylcellulose. 18 % showed clonogenic growth: growth rate from 1 to 62 colonies, depending on the case and on the addition of hydrocortisone and epidermal growth factor. The cells forming the colonies are fibroblasts or fibroblast-like.
The cells from 15 non-tumoral human effusions were also cultured in methylcellulose: In 20 % of cases colonies were formed. Before plating, immunological stainings with Mabs against cytokeratin, vimentin and macrophages, as well as the thymidine labelling index were performed on cell suspensions. The labelling and the mitotic index of stromal cell populations are not related to the formation of colonies in methylcellulose.

CMB 92

IDENTIFICATION OF GENES REGULATED BY EXPRESSION OF THE HA-RAS AND MOS ONCOGENES. A.K. Werenskiold, S. Hofmann and R. Klemenz, Ludwig Institute for Cancer Research, Bern Branch, Inselspital, CH-3010 Bern.
To study the molecular mechanisms that lead to cell transformation we attempt to isolate genes whose expression is influenced by the mutated human Ha-ras or the viral mos genes. We employ an inducible system (i.e., NIH 3T3 cells stably transfected with the oncogenes under the glucocorticoid inducible MMTV promoter). Proteins pulse labeled before or at different times after oncogene induction were analyzed on 2D gels. A few characteristic changes of the protein pattern are observed. Some but not all of the changes also occur after serum induction of quiescent cells. Degradation of vimentin is augmented and one tropomyosin is turned off. A 23 kd protein, which is strongly induced after oncogene activation, was also found to be induced during heat shock. It has been purified in reasonable amounts for sequence determination. We attempt to clone genes affected by the expression of the oncogenes. A cDNA library from oncogene expressing cells was constructed. 110,000 recombinants were analyzed by differential hybridization to radioactively labeled cDNAs representing the mRNA species from either induced or non-induced cells. 300 cDNA clones representative of mRNA species specific for ras-expressing cells have been isolated and are currently being further characterized.

CMB 93

INDEPENDENT EXPRESSION OF PLATELET-DERIVED GROWTH FACTOR (PDGF)-A AND PDGF-B mRNA
Powell, J.S., Sakariassen, K.S., Raines, E.W. and Ross, R., F. Hoffmann-La Roche & Co., Ltd., CH-4002 Basel and University of Washington, Seattle, WA, USA
Production of PDGF by cells of the vascular wall may be important in the process of atherosclerosis. We have investigated regulation of mRNA levels and PDGF-like activity in a recombinant cell line (BHK) and in human endothelial cells (EC) and smooth muscle cells (SMC) cultured from several vascular sites. PDGF-B mRNA, but not PDGF-A mRNA, increased in BHK cells after stable transfection with each of 4 human genes (up to 9 fold). PDGF-like activity also increased after culture in hypoxic conditions (5% O_2). For EC from saphenous vein, PDGF-B mRNA and PDGF-A mRNA were detected constitutively, but only PDGF-B mRNA increased (3 fold) during culture in 5% O_2 over 24 hours. For SMC, levels of PDGF-A mRNA did not change (arterial: 0.4 pg/µg total RNA and venous: 0.2 pg/µg) and no PDGF-B mRNA was detected during 72 hours in 5% O_2. Our data support the hypothesis that perturbation of certain cell types, including EC, may stimulate selective expression of PDGF-B. This mechanism may be a source of mitogenic activity in the development of intimal hyperplasia and lesion formation.

CMB 94

TUMOR NECROSIS FACTOR α (TNF) INDUCES ENDOTHELIAL CELL ELABORATION OF PLATELET-DERIVED GROWTH FACTOR (PDGF) ACTIVITY
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F. Hoffmann-La Roche & Co., Ltd., CH-4002 Basel, Switzerland, Oklahoma Medical Research Foundation, Oklahoma City, OK, USA and University of Washington, Seattle, WA, USA
Infiltration of the vessel wall by monocyte/macrophages is an early event in atherogenesis. This led us to examine the effect of the macrophage product TNF on the expression of PDGF activity by cultured human umbilical vein endothelial cells. Incubation with recombinant TNF results in five-fold increases in PDGF-A and PDGF-B transcription at 6h. The effect on PDGF-B is half maximal at 200 pM TNF and on PDGF-A at 3000 pM TNF. Exogenous PDGF-like activity is observed at 12h, and is about three-fold higher than the constitutive expressed baseline. TNF is detected by radioimmunoassay in human atherosclerotic plaques (about 6.0 pg/100 mg protein), but not in normal arteries. The presence of TNF in arterial lesions and the effect of TNF on PDGF-like activity in endothelium, lend support for a pathophysiological role for TNF in atherogenesis.

CMB 95

SPECIFIC BINDING OF ^{125}I-GRANULOCYTE-MACROPHAGE-COLONY STIMULATING FACTOR (GM-CSF) AND ^{125}I-INTERLEUKIN 3 (IL-3) TO MURINE HEMOPOIETIC CELLS

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We have shown that isolated murine granulocyte-macrophage progenitor cells can undergo up to 4 divisions in the absence of hemopoietic growth factors (CSFs). This apparently CSF-independent growth may represent stimulation by an existing pool of internalized CSF. To test this hypothesis, we have begun to examine the binding, internalization and degradation of GM-CSF and Il-3. Bacterially synthesized recombinant factors were iodinated by a 2-phase method, yielding biologically active preparations with specific activities in the range of 10^5 to $3x10^5$ cpm/ng of protein. Preliminary studies with the factor-dependent hemopoietic cell line FDC-P1, which responds to both factors, indicate that both labeled factors are specifically bound and that binding can only be competed for by the appropriate unlabelled factor. Using FDC-P2, which responds only to Il-3, ^{125}I-Il-3 but not ^{125}I-GM-CSF was bound. Specific binding to mouse bone marrow cells occurred with both factors. Binding of ^{125}I-GM-CSF to FDC-P1 and bone marrow cells was confirmed by autoradiography.

CMB 96

CHARACTERIZATION OF A HUMAN NEUROBLASTOMA CELL LINE THAT RESPONDS TO EPIDERMAL GROWTH FACTOR (EGF)

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A human neuroblastoma (NBL) cell line, CA-2E, has been established. The karyotype and antigen phenotype of the cells are compatible with a NBL origin. Colony formation in semi-solid medium by CA-2E cells at early passages (<20) is strictly dependent upon exogenous EGF. At later passages, the cloning efficiency in the absence of EGF increases, but the line remains sensitive to EGF, as evidenced by increased colony numbers and size. In short-term cultures with limiting concentrations of serum, EGF increases the proliferation rate of CA-2E cells. Long-term treatment (> 14 d) with EGF, on the other hand, results in a decreased rate of proliferation, primarily due to decreased numbers of clonogenic cells as detected in liquid and semi-solid media. Concomitant with decreasing proliferation rate, changes in morphology of the cells in culture were apparent. Treatment with EGF led to firmer attachment, increased spreading and, in some cases, elongated processes were evident. These results suggest that EGF may be a differentiation factor as well as a growth factor for CA-2E cells.

CMB 97

LAMIN PHOSPHORYLATION AND NUCLEAR ENVELOPE DISASSEMBLY INDUCED BY CHICKEN METAPHASE CELL EXTRACTS

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The nuclear lamina is a network of intermediate filaments lining the nucleoplasmic surface of the inner nuclear membrane. It is thought to serve skeletal functions important for nuclear envelope integrity and interphase chromatin organization. In chicken, three major lamins (termed lamins A, B_1 and B_2) have been described. During mitosis, when the nuclear envelope disintegrates, lamin filaments are disassembled, presumably as a consequence of hyper-phosphorylation. Using extracts prepared from metaphase-arrested chicken cells, we have been able to reproduce early mitotic events in vitro. Incubation of interphase nuclei in metaphase extracts resulted in disassembly of lamins and chromatin condensation in an ATP-dependent manner; no such effects were observed when using extracts prepared from interphase cells. Furthermore, metaphase extracts showed higher phosphorylating activities on lamin substrates than interphase extracts. To identify the mitosis-specific phosphorylation sites on lamin proteins, phospho-peptide analyses are currently in progress.

Physiology (PHY)

PHY 98

INHIBITION OF Ca^{2+}-ACTIVATION OF SKINNED PSOAS FIBRES BY THE PEPTIDE TnI (104-115)

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We have investigated whether a synthetic peptide comprising the amino acid residues 104 to 115 of troponin-I (TnI) (cf. J.E. van Eyk and R.S. Hodges: Biophys. J. 51, 240, 1987) inhibits the calcium-induced contractile responses of chemically-skinned rabbit psoas muscle fibres. Fibres were prepared by skinning with 1% Triton-X-100, then stored at -20°C in a mixture of glycerol and relaxing solution, and activated in a contraction solution. The peptide inhibited the isometric contractile responses elicited by 2.5 µM Ca^{2+} (IC_{50} 20 µM), and 50 µM caused a rightward shift of the calcium-force relationship by 0.3 pCa units, perhaps by inhibiting TnI/TnC interaction. A related peptide, mastoparan, had a similar, but weaker effect.

PHY 99

VERAPAMIL DELAYS ISCHEMIA INDUCED CELLULAR UNCOUPLING IN THE ARTERIALLY PERFUSED RABBIT PAPILLARY MUSCLE

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Intracellular(r_i) and extracellular(r_o) longitudinal resistance increase during myocardial ischemia. In this study we sought to show that the Ca++ antagonist, Verapamil (0.5µM), slows the onset of cellular uncoupling during myocardial ischemia. Cable analysis was performed utilizing the electrical parameters [amplitudes of the transmembrane action potential (ΔV_m) and extracellular bipolar electrogram (ΔV_o), the interelectrode distance (Δx) and the subthreshold current (I)] obtained from an arterially perfused isolated right ventricular papillary muscle preparation suspended in a H_2O-saturated atmosphere. Ten preparations were exposed to a Verapamil-free perfusate and 4 were exposed to Verapamil. After a 30 minute control period ischemia was induced by complete arrest of flow.

Results: Percent increase relative to pre-ischemia value after 23 minutes of ischemia (MEAN±SEM).

	Control	Verapamil	P-Value
Change r_i	95 39	0 18	< 0.05
Change r_o	34 ± 6	42 ± 17	< 0.05

Conclusions: (1) Verapamil delays the onset of cellular uncoupling during myocardial ischemia at a clinically relevant serum concentration (2) This delay can be attributed primarily to the slowing in the increase of intracellular longitudinal resistance (r_i).

PHY 100

MORPHOLOGICAL AND PERMEABILITY STUDIES OF REGENERATED RAT AORTA ENDOTHELIAL CELLS.

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The rat aortic model of endothelial injury (balloon catheter induced) has been exploited to study the accumulation and distribution of iodinated lipoproteins (IDL/LDL fraction) and albumin in de-endothelialized and re-endothelialized rat aorta. Concurrently, the development of cytoplasmic actin microfilament bundles (stress fibers) in regenerated endothelial cells was assessed, i) by immunofluorescent staining of "en face " preparations and ii) by morphometry, using thin section electron microscopy.
We have shown that regenerated endothelium covering intimal thickening (60 days after injury) contains more stress fibers than other areas of the injured or normal aorta. This coincides with the capacity of intimal thickening to retain IDL/LDL, but not albumin. Thus, regenerated endothelial cells covering thickened areas acquire new morphological features: it is conceivable that they represent a good marker of a dysfunctional or modulated arterial permeability.

PHY 101

EFFECTS OF SUBSTANCE P (SP),CALCITONIN GENE-RELATED PEPTIDE (CGRP) AND CAPSAICIN ON ELECTRICAL AND MECHANICAL ACTIVITY OF PIG CORONARY ARTERY IN VITRO.

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The effects of SP, CGRP and capsaïcin were studied on isometric tension and membrane potential of pig coronary arterial transversal strip in vitro. Both SP and CGRP relaxed the strip. The endothelium-dependent relaxation caused by SP was accompanied by a hyperpolarization whereas CGRP induced an endothelium-independent relaxation without any change in the membrane potential. Applied together, the mechanical effects of SP plus CGRP were additive. CGRP did not affect the hyperpolarization due to SP.
SP and CGRP are co-localized in nerve terminals of primary sensory neurones. They can be depleted by capsaïcin of their content in SP and CGRP. In order to examine a possible role of endogenous SP and CGRP, we applied capsaïcin. It contracted the strip. This contraction was adventitia-dependent, and inhibited by indomethacin (an inhibitor of the prostanoids synthesis).

PHY 102

NEITHER NITRIC OXIDE NOR NITROGLYCERIN ACCOUNT FOR ALL THE CHARACTERISTICS OF THE ENDOTHELIAL MEDIATED VASODILATATION.

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The endothelium derived relaxing factor (EDRF) is a labile diffusible agent that mediates relaxation of arteries caused by many vasodilators. It has been suggested that this factor could be nitric oxyde. Nitric oxyde (NO) is the active moiety of nitrovasodilators. Both NO and the nitrovasodilators produce vasorelaxation by the activation of the soluble form of guanylate cyclase. The relaxation caused by EDRF is characterized by a concomitant hyperpolarization of the smooth muscle cell membrane potential. Therefore we have tested whether the relaxation caused by NO and nitroglycerin on pig coronary arteries is likewise accompanied by a hyperpolarization. We show that NO and nitroglycerin relax smooth muscle without hyperpolarizing their cells. Moreover NO and nitroglycerin do not inhibit the electrical response of smooth muscle membrane potential evoked by the EDRF released by bradykinin (BK) or substance P (SP). We conclude that nitroglycerin and NO do not account for all the properties of the endothelial mediated vasorelaxation and that the hyperpolarizing action of the endothelium is not mediated by NO but by another molecule or mechanism.

PHY 103

THE RESISTIVE INDEX OF ARTERIAL VELOCITY-TIME-CURVES DEPENDS ON THE HEART RATE

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Recordings of arterial velocity-time-curves v(t) are clinically used for the early diagnosis of homograft rejection after kidney transplantation. It is known that immunological reactions cause an increase of peripheral resistance. The aim of this study was to investigate whether the Resistive Index RI = (max.syst.velocity - min.diastol.velocity)/max. syst.velocity is influenced by further parameters than the capillary bed impedance. The v(t) curves in the renal arteries of 3 pigs after duodeno-pancreatico-renal composite transplantation under immunosuppression were simultaneously monitored by implanted synchronized Doppler ultrasound transducers.
Results: a) Although the transplanted kidney is fed from the recipient iliac artery through the donor abdominal aorta the v(t) curves of the homolog and autolog arteries are identical. This could mean that RI does not depend on the circulatory properties proximal to the recording site.
b) RI depends on the heart rate HR. A proposition for calculating RI normalized for the average pig HR (93 min^{-1}) is presented. HR-independent RI = RI + 0.004·(HR-93)

PHY 104

REGULATION OF RENAL ERYTHROPOIETIN mRNA

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Erythropoietin (EPO) is a glycoprotein which is produced mainly in the kidney and whose production is stimulated in response to hypoxia. In order to correlate changes in secretion of EPO with changes in EPO mRNA content, serum EPO and EPO mRNA levels were determined in rats in response to varying degrees of normobaric hypoxia. Serum EPO levels were quantitated by scanning densitometry and normalized by hybridization with a control α Tubulin probe. Renal EPO mRNA was undetectable in control animals and also in animals exposed to a mild hypoxic stimulus (13.5% O_2). However, EPO mRNA content increased 1.5h after the initiation of a more severe hypoxic stress (7.5 and 10.5% O_2) and continued to increase until 4.5h of exposure. These increases correlated well with increases in serum EPO levels suggesting that the EPO response to normobaric hypoxia is mainly due to new synthesis of EPO and that this is a result of an increase in EPO mRNA content.

PHY 105

24 HOUR ENERGY EXPENDITURE (24-EE) AFTER WEIGHT LOSS IN OBESE SUBJECTS

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In 10 obese women, 24-EE was measured in a respiration chamber under 4 conditions: I) before weight loss (77.9 kg, 39% body fat); II) during weight loss (63.9 kg, 30.6 % body fat); III) following realimentation (62.5 kg, 30% body fat) and IV) 6-12 months later with "ad libitum" diet (68.8 kg, 34.5% body fat). The 14 ± 8 kg weight loss produced a decrease in 24-EE of 358 ± 272 kcal/d ($p < 0.01$), i.e. a decrease of 25 kcal/d·kg weight loss. The subsequent 24-EE (III and IV) remained lower than the value before weight loss. Values of postabsorptive respiratory quotient (RQ) were I) 0.81 ± 0.04; II) 0.76 ± 0.03 ($p < 0.001$), III) 0.81 ± 0.04, IV) 0.82 ± 0.04. A significant correlation between changes in RQ (III-IV) and the rate of body weight gain after cessation of the hypocaloric diet ($r = 0.81$; $p < 0.05$) suggests that postabsorptive RQ is a predictor of relapse of weight gain. An elevated postabsorptive RQ shows that less endogenous lipids are oxidized than when RQ is lower, the former condition favouring weight gain.

PHY 106

24 HOUR ENERGY EXPENDITURE (24-EE) IN PREGNANT WOMEN WITH A STANDARDIZED ACTIVITY LEVEL.

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24-EE and respiration quotient (RQ) as well as diet induced thermogenesis (DIT) were measured in a respiration chamber at 16 ± 1 (I), 28 ± 1 (II), 37 ± 1 weeks (III) of pregnancy (PG) as well as in the non pregnant postlactating period (NPPL) in 10 young women whose weight gain during PG was 11.3 ± 1.2 kg. With a standardized activity level (3 periods of 15 min walking on a treadmil, 10% slope, 2.6 km/h) 24-EE in the NPPL state averaged 2050 ± 77 kcal/d and increased to 2156 ± 68, 2353 ± 122 and 2423 ± 90 kcal/d ($p < 0.05$) from I to III respectively. Normalized for body weight 24-EE was nearly identical (34.8 to 35.5 kcal/kg.d). DIT was found to be reduced during PG (9 ± 1% vs 13.5 ± 1% in NPPL state, $p < 0.05$). 24 h RQ increased during I and II and progressively decreased throughout PG (0.92 ± 0.01, 0.91 ± 0.01, 0.89 ± 0.01 in period I to III vs 0.87 ± 0.01 in NPPL state, $p < 0.05$).
It is concluded that 24-EE increases during PG in proportion to weight gain, DIT is reduced, and the elevated RQ during the 2 first trimesters of PG is consistent with fat storage.

PHY 107

METABOLIC RESPONSES TO FLIGHT, FASTING AND FEEDING IN PASSERINES DURING MIGRATION

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Our preliminary results are part of a study concerning the physiological adaption of the energy metabolism in *Sylvia borin, S. atricapilla* and *Erithacus rubecula* during migration. Since serum levels of triglycerides (TG), uric acid (UA), β-hydroxybutyrate (β-OHB) and glucose (GL) are measured for the first time in free living birds, defined physiological conditions had to be analysed first. Fasting birds, which were kept inactive overnight (a) were compared with birds caught during flight (b) and birds feeding in resting grounds (c). The 3 conditions are metabolically characterized by: Low GL and high β-OHB levels in (a) and (c), distinguish fasting from feeding birds; high UA levels in (b) and (c) separate active from inactive, fasting birds. The circadian variation in serum levels of resting birds reflect the feeding behaviour and energetic needs: GL shows a steep increase between 5^{00} and 8^{00}, whereas TG and UA have their peak around midday. In accordance with these results β-OHB was found early in the morning only. During night migration a continuous GL decrease could be measured.

PHY 108

EFFECTS OF PRIMING EXERCISE ON $\dot{V}O_2$ KINETICS AND O_2 DEFICIT.

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$\dot{V}O_2$ kinetics and increase of blood lactate (ΔLa) concentration were determined on six moderately trained subjects during cycling (C) or stepping (S) square wave transitions from lower (rest (R) to 60% $\dot{V}O_2$max) to heavier (40 to 85% $\dot{V}O_2$max) loads. In C, the $t_{\frac{1}{2}}$ of the $\dot{V}O_2$ on- response increased monotonically from 22 to 63s with increasing the $\dot{V}O_2$ baseline from R to 60% $\dot{V}O_2$max. On the contrary, in S, the fastest $\dot{V}O_2$ kinetics ($t_{\frac{1}{2}}$ = 13s) was attained when starting from a $\dot{V}O_2$ baseline = 20% $\dot{V}O_2$max, the $t_{\frac{1}{2}}$ being longer (25s) when starting from R or from 50% $\dot{V}O_2$max. The slower $\dot{V}O_2$ kinetics in C was associated with much greater ΔLa values. The present findings are consistent with the hypothesis that the O_2 deficit incurred at the onset of a rectangular exercise is the sum of three terms: 1) body O_2 stores depletion, 2) early La production, and 3) high energy phosphates breakdown. The relative magnitude of these terms (especially early La) can vary markedly in different exercise modes and/or conditions (e.g. posture, arms vs legs muscles, training status, blood perfusion) leading necessarily to changes of the $\dot{V}O_2$ on-kinetics.
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PHY 109

INCREASE OF VENTILATION DURING ELECTRICALLY EVOKED MUSCLE CONTRACTIONS IN HUMANS

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The importance of the different nervous and humoral stimuli for the exercise hyperpnea is still a matter of debate. Therefore, we analysed ventilation on-line breath-by-breath during electrically evoked muscle co-contractions of the lower trunc and the thighs in 19 subjects (21 - 27 years old). The intensity of the electrical stimuli were individually determined by the subjects to avoid intensities causing discomfort. Stimulation lasted 12 minutes. The electrically evoked muscle co-contractions led to a steep increase in ventilation from 7.3 $\pm$ 1.6 to 12.9 $\pm$ 4.0 l/min within the first minute in 9 responders. After another minute, ventilation slowly decreased reaching a final steady state of 10.5 $\pm$ 3.5 l/min in contrast to the initial steep (neurogenic) and then slower (humoral) increase in ventilation generally observed at onset of exercise. The remaining 10 subjects did not substantially increase ventilation.

PHY 110

AUTONOMIC MECHANISMS OF AIRWAY NARROWING AND INCREASED BRONCHIAL REACTIVITY AT NIGHT

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Recently we pointed to the impact of parasympathetic lung innervation for the day/night variations of airway calibre. In the present study we complementarily investigated the role of the β-adrenergic system in the circadian rhythms of bronchial resistance and reactivity. In 10 selected healthy subjects (non-smokers and non-atopics) heart rate (HR) and - by body plethysmography - specific airway conductance (SGaw) as well as bronchial reactivity to aerosolized histamine (bRH) were measured in intervals of 4.8 h during 2x24 h, i.e. one day without, the other with 280-560 mg/day propranolol. Propranolol significantly reduced HR by 6% (280mg) and 9% (560mg), while neither dosage significantly affected the mean values of SGaw und bRH. Group cosinor analysis revealed clear circadian rhythm in SGaw (peak time 17.03, rhythm amplitude 22% of mean) and in bRH (peak time 01.54, rhythm amplitude 16%); with propranolol these day/night-variations were detected without changes in peak time and rhythm amplitude. Conclusions: β-receptor blockade in selected healthy humans did not affect circadian rhythms of airway calibre and bronchial reactivity. Thus increased vagal tone rather than reduced β-adrenergic activity accounts for the nocturnal airway narrowing.

PHY 111

THE RELATIVE ROLE OF VENTILATORY VARIABLES IN ELICITING APNEA DURING HIGH-FREQUENCY OSCILLATORY VENTILATION (HFOV)

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The optimal choice of ventilatory variables (pump frequency = fP, stroke volume = Vs, mean airway pressure = Paw, amplitude of pressure swings = Posc) in clinical application of HFOV is necessary to ensure adequate gas exchange with minimal risk for the patient. However, also the impact of each of these variables on vagal lung receptors and thus on the control of breathing has to be considered. We therefore analysed the role played by fP, Vs, Paw and Posc in the changes of spontaneous breathing pattern in rabbits anaesthetized by urethane. Three types of respiratory responses to HFOV were observed: (a) decrease of breathing frequency, (b) increase of breathing frequency and (c) apnea accompanied by a tonic diaphragmatic activity. Type (a) occurred at low fP and Vs independently on Paw and Posc. Type (b) occurred at high fP and Posc, whereas the incidence of type (c) increased with both increasing Paw and Posc. From the results we conclude that , depending on ventilatory variables used, different groups of vagal receptors are stimulated giving rise to different respiratory responses to HFOV.

PHY 112

RIA DATA ANALYSIS: SIMPLE IS BEST

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The most current way of processing the RIA data today is to construct a standard curve displaying the log of the (B/Bo)/(1-(B/Bo)) ratio (the so-called logit (B/Bo)) versus the log of the cold ligand. However, if a) the hot ligand saturates the anti-ligand antibody to a reasonable amount (90%), and b)the tracer has the same affinity for the antibody as the cold one, a straight line is obtained by plotting the Bo/B ratio versus the actual ligand concentration, a formula that accounts mathematically for the relationship between the bound cpm and the cold ligand concentration. Lack of linearity can result from insufficient antibody saturation by the tracer, or from different affinities of the tracer and the cold ligand for the antibody; the data reduction has to be achieved using a polynomial transform, a spline transform or equivalent. The logit-log transform, or any other linear transform, will not straighten a curve based on experiments where conditions a) and/or b) are not satisfied. When a) and b) are satisfied however, the availability of electronic data processing devices does not justify the introduction of useless transforms, such as the two logarithmic transforms involved in the logit-log formula.

PHY 113

SUBTHERAPEUTIC INSULIN DOSES CONTINUOUSLY DELIVERED BY OSMOTIC MINIPUMPS COUNTERACT HYPOTHALAMO-PITUITARY-GONADAL ALTERATIONS IN STREPTOZOTOCIN (SZ)-DIABETIC RATS.

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In animal models gonadal axis lesions are probably responsible for diabetic reproductive disorders. To test the pathogenic role of insulin deficiency, we studied the hypothalamo-pituitary-gonadal axis of insulin-treated (by osmotic minipump) SZ-diabetic, untreated diabetic, and control rats. Further, to determine whether possible insulin effects are limited to the gonadal axis or generally acting, we have also studied for comparison the retinal microangiopathy. The hypothalamo-pituitary-gonadal axis of insulin-treated diabetic animals was almost unchanged. On the contrary, the retinal microangiopathy was only slightly influenced by subtherapeutic insulin doses. In conclusion, subtherapeutic insulin doses counteract most of the effects of diabetes on the gonadal axis. Thus, the gonadal axis impairment in SZ-diabetic animals may be related to the fall of plasma insulin below a critical level. Further, the various organ systems may respond to different plasma insulin threshold levels.

PHY 114

ROMAN LOW (RLA) AND HIGH (RHA) AVOIDANCE RATS: INVERSE CORRELATION BETWEEN PITUITARY AND PINEAL WEIGHTS, AND EFFECT ON SEXUAL MATURATION

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Sexual maturation was studied in two psychogenetically selected rat lines (RLA and RHA). Organ weights, physiological and endocrinological parameters were evaluated at 31, 35 and 41 days of age in males and females, and after 3 estrous cycles in females. RLA male rats had smaller seminal vesicles with increasing age when compared to RHA rats, while RLA females had irregular estrous cycles. Pituitary weights at all ages were higher in RLA males and females, while pineal weights were smaller ($p<.001$). There were no difference between RLA and RHA rats in their pituitary content of LH, nor in their plasma levels of LH. Pituitary content of FSH and prolactin (PRL), and pituitary GnRH binding varied according to sex and age between both types of rats, while plasma PRL was higher at all ages and in both sexes in RLA rats. Pineal contents and plasma levels of melatonin were lower in RLA rats. These results describe an inhibition of sexual maturation in RLA rats which could be due to the association of high PRL levels and low melatonin production.

PHY 115

CALCIUM-DEPENDENT ENERGY EXPENDITURE IN THE RELAXED SOLEUS MUSCLE OF THE MOUSE

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Steady rates of oxygen consumption and heat production by non-contracting muscles were measured before and after decreasing cytoplasmic calcium activity (Ca^{a}_{cy}) with dantrolene (10^{-5}M) or high extracellular Mg^{++} (11 mM), or after increasing Ca^{a}_{cy} by doubling extracellular potassium. Factorial experiments were designed to study the effects of cold acclimation of the donor animal (5 vs. 33 °C), of the addition of 5 mM succinate to the glucose-containing Krebs-Ringer perifusate, and of raising measurement temperature (33 vs. 23 °C). Protocols were tested for constancy of muscle tension, cytoplasmic pH and the respiration-derived energy stores. Total rate, and calcium-dependent fraction (8-13%), of basal energy dissipation were enhanced by cold acclimation (+ 25%) and added succinate (+ 24%) independently. Minimum calcium-dependent fraction of total energy dissipation became 30% (cold-acclimated) and 24% (control) upon threshold-contracture depolarisation. Succinate potentiated the effect of raising measurement temperature on both total and calcium-dependent dissipations. These results suggest that resting muscle metabolic rate is substrate-limited and that the energy cost of Ca^{a}_{cy} homeostasis increases significantly upon Ca^{a}_{cy} changes inducing < 3% increments of resting tension.

PHY 116

POLARIZED Na^+/H^+ EXCHANGE AND ITS REGULATION BY PARATHYROID HORMONE (PTH) IN CULTURED OPOSSUM KIDNEY (OK) CELLS

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Intracellular pH (pH_i) of OK cells was measured by fluorometry using 2'7'-bis-(2-carboxyethyl)-5,6 carboxyfluorescein (BCECF). In suspended cells which have been acid loaded with NH_4Cl prepulses, recovery from an acid load is Na^+-dependent and sensitive to 5 µM of the amiloride derivative, ethylisopropylamiloride. The rate of acid extrusion is tightly controlled by pH_i and is quiescent above pH_i values of 7.1/7.2 (set point). Na^+-dependent recovery of pH_i from acid loads is inhibited by incubation (15 min) with PTH at concentrations greater than 10^{-10} M; mediated intracellularly by cAMP. The extent of PTH inhibition is higher when Na^+/H^+ exchange is studied at lower pH_i values, suggesting that it is not related to a shift in set point. In single cell measurements of cells in a monolayer, ordered additions of Na^+ to the basolateral and apical superfusion solutions revealed that at least 90 % of the Na^+-dependent pH_i recovery is localized in the apical membrane. It is concluded, that apical Na^+/H^+ exchange is involved in pH_i control in OK cells and that it is controlled by PTH (supported by SNF 3.881.085).

PHY 117

PRETRANSLATIONAL CONTROL OF Na^+-P_i COTRANSPORT IN CULTURED OPOSSUM KIDNEY (OK) CELLS

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Na^+ phosphate (P_i) cotransport in OK cells is regulated by PTH (parathyroid hormone) and phosphate deprivation. Recovery from PTH inhibition and the adaptive response (increase) is prevented by inhibitors of protein synthesis at the translational level (cycloheximide) but not by inhibitors of transcription (actinomycin D). Cordycepin (3-deoxyadenosine) but not other deoxynucleotides inhibited the increase in Na^+-P_i cotransport (PTH recovery/adaptation) suggesting a specific role of mRNA processing mechanisms (eg. polyadenylation). We have now isolated total poly A(+)RNA from control and P_i-deprived cells. Injection of total poly A(+)RNA (50 ng/oocyte) into Xenopus Laevis Oocytes leads to a ~2,5-fold increase in Na^+-dependent P_i transport by the oocyte. This strategy together with in vitro translation assays is now used to analyze size-fractionated poly A(+)RNA for mRNA encoding for a protein required for Na^+-P_i cotransport. Furthermore, it will allow to test the hypothesis of control of Na^+-P_i cotransport at a posttranscriptional (eg. polyadenylation) but pretranslational level (supported by SNF 3.881.085).

PHY 118

FUSION OF GANGLIOSID-CONTAINING LIPOSOMES WITH INFLUENZA VIRUS-INFECTED CELLS

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Ganglioside-containing liposomes with various lipid compositions were produced with a controlled detergent dialysis procedure (Zumbühl and Weder, 1981, Biochim. Biophys. Acta 640, 252-262), which reproducibly yields a homogeneous population of liposomes. The influence of gangliosides and various lipids on the fusion activity of these liposomes was studied. As fusion partners we used purified PR8 influenza viruses or influenza virus-infected MDCK (Madin Darby canine kidney) cells. Fusion was measured by incorporation of octadecyl Rhodamine B chloride (R18) into liposomes at highly quenched concentrations (>8% w/w). Upon fusion the fluorescent compound is diluted into the membrane of the fusion partner, i.e. the virus or virus-infected cell, leading to dequenching, which can be quantitated by fluorescence spectroscopy. Ganglioside- containing liposomes with significant fusion activity are of great potential interest for specific drug delivery to virus-infected cells.

PHY 119

IN VITRO GASTROINTESTINAL TRANSPORT OF IRON AS A MODEL FOR ACTINIDE UPTAKE

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Biology and Environment, 81/SU, EIR, CH-Würenlingen

Although the chemistry of actinides is quite different from any stable metal ion, plutonium, americium and neptunium are bound with high affinity to iron binding proteins, leading to an accumulation of actinides in iron storing organs such as the liver. Therefore, and for similarities in solubility at a neutral pH and the possibility of different oxidation states, iron may be used as a model for the behaviour, i.e. uptake, of the poorly soluble actinides in biological systems. With our in vitro system we can compare ferric iron uptake with uranium uptake into brush border membrane vesicles. Our aim is to elucidate the role of the transmembrane iron transport system in the GI-uptake of actinides.

PHY 120

STUDIES ON SODIUM TRANSPORT AND ITS HORMONAL REGULATION IN PRIMARY CULTURES OF BOVINE TRACHEAL EPITHELIAL CELLS.

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Epithelial cells were isolated from the bovine trachea by collagenase treatment and grown under standard conditions. After a 6 to 8 days incubation, the cells reached confluence and showed a typical aspect for an airway epithelium, with domes formation and a large number of beating cilia. The time courses for uptake and wash out of $^{22}Na^+$ were measured. The half time for isotope equilibration was close to 4 min. Net uptake was linear with time for 2 min. and the influx, taken as the initial slope, was 84 ± 9 nEq min^{-1} mg $prot^{-1}$ (mean ± SEM, n = 9); it was sensitive to amiloride and, to a lesser extent, to bumetanide. For Na^+ wash out, the half time was close to 5 min.; it was shifted to 10 min. following ouabain treatment. Aldosterone, 1 µM, and dexamethasone, 1 µM, added for either 8 days or 18 hours enhanced Na^+ uptake; this effect was suppressed by amiloride. Isotope equilibration curves indicated that the Na^+ content of the cells was increased by 20 % following corticosteroid administration. An analysis of wash out curves supports the view that steroid-stimulation of Na^+ transport is accomplished by concerted adjustments of both influx and efflux.

PHY 121

CARBON DIOXIDE PRODUCTION OF THE CHICK EMBRYO FROM LAYING TO NEURULATION.

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The CO_2 production ($QbCO_2$) of the chick blastoderm has been determined in vitro using a non-invasive conductometric micromethod. The mean values of $QbCO_2$ were 16 ± 6, 153± 45 and 231 ± 43 nmol/h at laying, gastrula stage and neurula stage, respectively. Up to gastrula, $QbCO_2$ values were independent of exogenous glucose but at neurula stage $QbCO_2$ value decreased by about 30 % in glucose-free medium. The suppression of the contractile activity resulted in a 56 % decrease of $QbCO_2$. Interestingly, the respiratory exchange ratios (R) at laying and gastrula stage were much higher than 1 (i.e. 3 and 1.7, respectively), irrespective of glucose, while R was equal to 1 at neurula stage. Our results indicate that in the embryonic tissue (1) unlike in differentiated tissue, a valid information about the character of the oxidized substrates cannot be drawn from the R values because (2) a large extra-mitochondrial source of CO_2 is present in the tissue and (3) the relative proportions of the CO_2 producing pathways vary throughout the early development.

PHY 122

SODIUM CURRENT CLOCK IN THE EARLY CHICK EMBRYO

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Voltage clamp experiments were done in the gastrulating chick blastoderms. Short circuit current, transectodermal potential (interior positive) and total resistance were 8 μAcm^{-2}, 2mV, 200 Ωcm^{-2} for the embryonic area pellucida and 22 μAcm^{-2}, 9 mV and 500 Ωcm^{-2} for the extraembryonic area opaca.

The current (appKm:25 $mmoll^{-1}$) is entirely due to active pumping of sodium across the ectoderm. Sodium entry into the cells depends on glucose and bicarbonate uptakes and is sensitive to amiloride. In the area opaca, the sodium current and transectodermal potential show regular oscillations with period of 3 to 5 min and amplitude of + 5 to 10% of the mean value. The oscillations are abolished by removal of glucose and bicarbonate.

This oscillating and radially organized pattern of electrochemical activity might represent a primitive system of intercellular communications governing the ordered cell migrations in the early embryonic development. **SNSF Grant No.3.418-0.86**

PHY 123

SEASONAL RHYTHMS IN BLOOD PRESSURE, BLOOD GLUCOSE AND PUPIL SIZE IN HEALTHY SUBJECTS

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To elucidate possible long-term adaptive processes related to the seasons, psychobiological variables investigated in depression were measured monthly throughout an entire year in 10 healthy volunteers (N=6♀, 4♂; mean age: 39,3 ± 9.68 y, range 24-59 y; 8h, 16h). No seasonal changes were found in heart rate, oral temperature, body weight and self-ratings of mood and fatigue, whereas blood pressure, blood glucose and pupil size varied significantly. Blood pressure was highest in autumn/winter with a nadir in May; blood glucose was lowest in autumn compared with all other seasons, with higher am:pm values, that were inversed in spring. Dark-adapted pupil size was smallest in February, largest in August, with the greatest am:pm difference in autumn. Similar findings have been reported for visual sensitivity, demonstrating a steady decline in liminal values from August to February (Sweeney et al., 1960) and a maximal day/night difference in autumn (Bassi et al., 1987). That pupil size was smallest in winter when natural light exposure was least, and largest in summer when greatest, indicates a different regulatory mechanism in long-term as opposed to short-term light response.

Biochemistry (BIO)

BIO 124

THE THREE-DIMENSIONAL STRUCTURES OF METALLOTHIONEIN-2 FROM RAT AND RABBIT DETERMINED BY NMR IN SOLUTION

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The spatial structures of $[Cd_7]$MT-2 from rat and $[Cd_7]$MT-2a from rabbit were determined by NMR in aqueous solution. The two homologues have 11 substitutions in the sequence of 62 amino acids but nearly identical conformations. These, however, differ markedly from the published crystal structure of rat $[Zn_2,Cd_5]$MT-2 (Furey et al., Science 231,704(1986)). The determination of the solution conformations involved the following steps: (i) Reconstitution of apo-MT2 with ^{113}Cd to obtain $[^{113}Cd_7]$MT2. (ii) Complete sequence-specific assignments. (iii) Determination of the connectivities between the ^{113}Cd-ions and the Cys residues with heteronuclear $[^{113}Cd,^1H]$-COSY. (iv) Collection of upper limits on intramolecular ^1H-^1H distances with homonuclear ^1H-NOESY. (v) Distance geometry calculations of the three-dimensional structure, using the ^{113}Cd-Cys connectivities and the ^1H-^1H NOE distance constraints as input for the program DISMAN.

BIO 125

PURIFICATION OF PREGNANCY-ASSOCIATED PLASMA PROTEIN B (PAPP-B): NEW LIGHT ON A LONG NEGLECTED PROTEIN

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In the last twenty years pregnancy- specific and pregnancy-associated proteins have gained increasing importance.PAPP-B has to belong to the latter group as long as placental/fetal specifity is proven, but no more work has been undertaken since Lin et al. described it in 1973. The knowledge of the physico-chemical properties of this protein is very limited: Mr=1,000,000,pI=4.6-5.0,electrophoretic mobility β_1. We used late pregnancy serum or homogenised placenta as source materials, starting with an ammonium sulphate salting out at pH 5.0 and 30% saturation. The precipitate was bound to DEAE-Sepharose at pH 5.5 and eluted with a stepwise NaCl gradient. The 0.3 M NaCl fraction was concentrated and subjected to gel filtration on Sepharose 2B. PAPP-B activity was detected by rocket- or counter-immunoelectrophoresis using polyclonal rabbit antiserum kindly supplied by Dr. Lin. PAPP-B positive fractions were at last purified by a chromatofocusing step. The elution pattern contained a sharp major peak at pI 4.8. The protein yield of the preparation was in the order of 10 E-4 %. On the isoelectric focusing a sharp band at pI 4.7 was seen together with two more weak ones at pI 4.6 and 5.0. Counter- immunoelectrophoresis gave two precipiting lines.

BIO 126

HUMAN COMPLEMENT COMPONENT C1s. PARTIAL SEQUENCE DETERMINATION OF THE A-CHAIN AND IDENTIFICATION OF DISULFIDE BONDS.

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Human C1s proenzyme was isolated by a rapid two-stage medhod, involving affinity chromatography on IgG-Sepharose and ion-exchange chromatography on DEAE-Sephacel. C1s was activated by C1r and after reduction and alkylation the A-chain was isolated on DEAE-Sephacel. Fragments of the heavy chain were generated by chemical and enzymatic cleavages for sequence analysis.
To identify disulfide bridges, the unreduced proenzyme was digested with pepsin and generated fragments were purified by reversed phase HPLC. Cystine containing peptides were detected with a fluorometric thiol specific reagent. Disulfide placements were facilitated by direct identification of PTH-cystine during Edman degredation of paired fragments.

BIO 127

CRYSTALLOGRAPHIC DATA FOR LIGNIN PEROXIDASE

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Phanerochaete chrysosporium is a wood-degrading white-rot fungus. It produces several extra-cellular peroxidases which are directly involved in lignin degradation. One of the haem-containing isozymes, glycosylated, M_r= 40 kDa, was purified by isoelectric focussing and crystallized from polyethylene glycol (using hanging drop and vapour diffusion). The needle shaped crystals have a maximum size of 0.15x0.3x1.0 mm. They appear brown in color and exhibit absorption bands characteristic for the haem group. Diffraction studies show that the lignin peroxidase crystals are orthorhombic, space group $P2_12_12_1$, with unit cell dimensions a=41.6 Å, b=73.0 Å, c=109.5 Å. The asymmetric unit contains a single peroxidase molecule. A native data set to 2.9 $Å^{-1}$ resolution has been collected with synchrotron radiation at DESY (Hamburg, FRG). These data have been processed.

BIO 128

HIGH RESOLUTION DATA FROM CRYSTALS OF ALDOLASE

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The glycolytic enzyme aldolase splits fructose-1,6-bisphosphate into dihydroxyacetone phosphate and glyceraldehyde-3-phosphate. The enzyme from Drosophila melanogaster is a homotetramer, M_r=158 kDa. We have shown that its catalytic properties change with temperature: the Arrhenius plot of the invertebrate enzyme has a break at 18 °C, seemingly reflected in the behaviour of the crystals too. Aldolase crystals from Drosophila are less sensitive to irradiation and ambient temperature than those from other species. We have collected native data to 2 $Å^{-1}$ (space group $P2_12_12_1$: a=86.3 Å, b=115.6 Å, c=151.4 Å, Z=4) and two derivatives (platinum, 2 $Å^{-1}$; gold, 2.8 $Å^{-1}$) at +4 °C at a synchrotron (Daresbury/UK). These data are currently processed (MOSCO and CCP4 program packages). The R-factors obtained are highly acceptable. Two new, monoclinic, crystal modifications have been found for Drosophila aldolase modified at the active site. They are closely related to the orthorhombic native crystals.

BIO 129

ISOLATION AND CHARACTERISATION OF THE γ-BUTYROBETAINE BINDING PROTEIN FROM AGROBACTERIUM SP.

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The γ-butyrobetaine binding protein involved in the transport of γ-butyrobetaine in Agrobacterium sp. has been isolated and purified by chromatographic and electrophoretic techniques. This protein binds its substrate (γ-butyrobetaine) with a K_d of 1 uM. The protein electrophoresed in non denaturing conditions in the presence of its radioactive ligand, showed the migration of the undissociated complex. When the experiment was performed in the presence of additional amount of non radioactive substrate, the label was released. The binding protein has a molecular weight of 52000 and an isoelectric point of 4.2. Amino acid analysis showed a high content of acidic residues, consistent with the pI, and the absence of cystein.

BIO 130

3-DIMENSIONAL STRUCTURE OF THE CYTOKINE INTERLEUKIN-1ß

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Interleukin-1ß (IL-1ß) is a 17kDa cytokine which produces a variety of biological responses, including lymphocyte proliferation and fever. We have crystallised high-purity human recombinant IL-1ß (Biogen AG, Geneva) by the hanging drop method from ammonium sulphate solutions. The crystals belong to space group P4$_2$ with unit cell dimensions a=b=54.9Å, c=76.8Å. There is one molecule per asymmetric unit implying a crystal solvent volume of 63%. Diffraction data to a resolution of 3.0Å was collected by diffractometry. Phases for the native structure factors were derived from two heavy atom derivatives supplemented by solvent flattening techniques. A 3.0Å electron density map was calculated. With the aid of a graphics display system, the entire known amino acid sequence has been fitted to the electron density. IL-1ß is composed of 12 anti-parallel ß-strands which are connected by reverse turns and loops. There are no helices. The structure has 3-fold structural pseudosymmetry which does not seem to correspond to any strong homology in the amino acid sequence. Structure refinement of IL-1ß is well underway.

BIO 131

SEQUENCE-SPECIFIC ^{1}H NMR ASSIGNMENTS AND DETERMINATION OF THE SECONDARY STRUCTURE OF α-NEUROTOXIN FROM DENDROASPIS POLYLEPIS POLYLEPIS IN AQUEOUS SOLUTION.

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Sequence-specific resonance assignments of the proton NMR spectrum of α-neurotoxin in aqueous solution at pH 4.0 were obtained at 20°C and 36°C, using the COSY, RELAYED-COSY and NOESY techniques. All backbone protons were assigned, with the exception of those of Ser 8, the amide proton of His 32 and the C^{α} proton of Glu 38. Assignments were also obtained for most of the side chains. Proton exchange kinetics were measured in D_2O at 20°C and used to identify the slowly exchanging amide protons. Tight turns were located from the sequential connectivity patterns and the NH-C^{α}H coupling constants. Together with long-range NOE's these data define the secondary structure. The global architecture of the protein is dominated by an antiparallel β-sheet with three strands consisting of the residues 23-31, 34-42 and 52-56.

BIO 132

^{1}H NMR STUDY OF RECOMBINANT DESULFATOHIRUDIN FROM YEAST

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The three-dimensional structure of recombinant desulfatohirudin, which is a derivative of the specific thrombin inhibitor hirudin, was determined by ^{1}H NMR. Using 2D NMR experiments at 500 MHz, sequence-specific assignments were obtained. Upper bounds on ^{1}H-^{1}H distances were determined by NOESY. The three disulfide bridges were found at the same positions as in natural hirudin. Four hydrogen bonds were identified by amide proton exchange rate measurements. In all, these experiments provided 208 distance constraints. This input for the distance geometry calculations with the program DISMAN was supplemented by 19 Φ-angle constraints derived from NH-αH coupling constants. So far, five structures were computed, with average violations of the distance constraints smaller than 0.04 Å/constraint, and an average pairwise RMSD for the polypeptide backbone of 2.4 Å. The conformation is characterized by two antiparallel β-sheets connected by a type II turn, and anchored in space relative to each other by two disulfide bridges. There is extensive similarity between natural hirudin and the recombinant protein. Additional structure refinements will be needed to explore potential local differences between the two molecules.

BIO 133

STRUCTURAL INVESTIGATIONS ON THE B740-ANTENNA COMPLEX OF CHLOROFLEXUS AURANTIACUS

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Formation of chlorosomes, oblong vesicular bodies attached to the cytoplasmic membrane, is one of the most prominent characteristics of green photosynthetic bacteria. Volume and number of chlorosomes increase with decreasing light intensities, the specific absorption range of chlorosomes endows green bacteria with an ecological niche among other photosynthetic organisms.
The present study aims at exploring the structural organi=zation of the B740-antenna complex, located in the chloro=somes of Chloroflexus aurantiacus.
Chlorosomes were prepared out of a membrane fraction by treatment with 2% miranol and 7M urea. Exposure of washed chlorosomes to low concentrations of nonidet-P40 apparently resulted in release of a Bchlc-protein complex. Subsequent purification was obtained by gel filtration on FPLC Super=ose 6 or Fractogel HW-55.
Several properties of the pigment-protein complex will be discussed.

BIO 134

PROTEASES SOLUBILIZE MEMBRANE-BOUND CHOLINE ACETYLTRANSFERA-SE

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In the cholinergic nerve endings from *Torpedo* electric organ an amphiphilic form of choline acetyltransferase (ChAT) seems to be specifically associated to the synaptosomal plasma membrane (SPM). In order to understand the mode of attachment of this enzyme to SPM, purified and previously washed SPMs (prot. conc. 0.5mg/ml) were incubated with a selected protease (5µg/ml) for 30 min. at 22°C. Then the procedure described by Futerman *et al.* (Biochem. J. *226*(1985)369) was followed. Proteinase K, trypsin, pronase and thermolysin were tested. Thermolysin had no effect. Proteinase K was the most efficient: a recovery of about 90% of initial ChAT activity was obtained, 65% in the supernatant and 25% in the SPMs pellet. Trypsin (tot. 99%, s. 58%, p. 41%) and pronase (tot. 97%, s. 48%, p. 49%) were also effective. Moreover, solubilization by these proteases was concentration and time dependent. Two phospholipases were also tested. Phosphatidylcholine-specific phospholipase D from Savoy cabbage had no effect and phosphatidylinositol-specific phospholipase C from *Bacillus cereus* solubilized only the acetylcholinesterase activity from SPMs. These data are interesting, but purified SPMs always contain high amounts of detergent-insoluble ChAT activity.

BIO 135

A NEW POSSIBLE BINDING SITE FOR BACTERIOCHLOROPHYLL B WAS FOUND IN A LIGHT HARVESTING POLYPEPTIDE OF E. HALOCHLORIS

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Ectothiorhodospira halochloris is an extremely halophilic, photosynthetic bacterium, containing bacteriochlorophyll b like Rhodopseudomonas viridis. In vivo, this pigment is non-covalently bound to individual antenna polypeptides, forming specific antenna complexes. Whole cells from E. halochloris were extracted with an organic solvent mixture. At least 4 light harvesting polypeptides (LHP) were purified by gelfil-tration on LH-60 and consecutive FPLC RP-Chromatography. The complete amino acid sequence of the 7.4 kD polypeptide was determined. The polypeptide shows a three domain structure indicative of an integral membrane protein, similar to the structure of the LHP's from other purple bacteria. Sequence homologies to the α-LHP's of purple bacteria range from 23.5 to 36.4%. The typical His residue, identified in all antenna polypeptides of purple bacteria assigned as the possible bacteriochlorophyll binding site, was found to be replaced by asparagine.

BIO 136

A POLY (ALA-PRO) PEPTIDE LINKS TWO FUNCTIONAL DOMAINS OF THE III^{man} SUBUNIT OF THE BACTERIAL MANNOSE PERMEASE

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The mannose permease of E. coli catalyzes sugar transport concomitant with sugar phosphorylation. It consists of two transmembrane subunits ($II\text{-}P^{man}$, $II\text{-}M^{man}$) and a membrane associated cytoplasmic subunit (III^{man}). III^{man} is composed of two domains which are linked through a hinge peptide consisting of Ala-Pro repeats and a few Lys. The two domains can be separated by limited proteolysis of the hinge with trypsin. Both domains are transiently phosphorylated at a histidyl residue, and phosphoryl transfer between the two centers is reversible. The N terminal domain is phosphorylated by a cytoplasmic high-energy phosphoryl carrier protein. The C terminal domain catalyzes phosphorylexchange between mannose and mannose 6-phosphate at equilibrium. To further characterize the function of the Ala-Pro hinge as a linker between protein domains, a number of changes have been introduced into this region by site-directed mutagenesis. Their effects on the catalytic function of III^{man} and the mannose permease will be discussed.

BIO 137

CYSTEINE BOND PATTERN OF COMPLEMENT COMPONENT C8γ AND ITS STRUCTURAL HOMOLOGY TO PLASMA PROTEIN HC

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Anti-C8α-γ specific antibodies were used to isolate cDNA clones from a human liver expression library. Antibodies affinity-purified on the expressed hybrid protein of one clone bound exclusively to the γ-chain of reduced C8α-γ. This clone, as well as a second full length cDNA clone obtained by hybridization screening, were sequenced and the complete primary structure for C8γ was established. Cyanogen bromide cleavage of C8α-γ released a 12 kDa carboxy-terminal C8γ fragment under both reducing and nonreducing conditions which was identified by fragment-specific, affinity-purified antibodies. Our data clearly show that C8γ has one internal disulfide bridge between cys-76 and cys-168 within the carboxy-terminal 12 kDa fragment, whereas the remaining cysteine residue 40 forms the disulfide bridge with C8α. The overall sequence homology to plasma protein HC (23% amino acid identities) and the conservation of one internal cysteine bond and one free, surface-located cysteine residue suggests a highly conserved three-dimensional structure of C8γ and protein HC and also a possible functional relationship between these proteins.

BIO 138

CYTOCHROME C OXIDASE FROM PARACOCCUS DENITRIFICANS: BOTH HEMES ARE LOCATED IN SUBUNIT I

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The two subunit cytochrome c oxidase from P. denitrificans has been sequentially digested with chymotrypsin and Staphylococcus aureus V8 protease. The enzyme smaller subunit was split into peptides which were eliminated by anion exchange high performance liquid chromatography. The larger subunit was only digested to a limited extent (from an apparent molecular weight of 45000 to 43000) and conserved spectral properties not distinguishable from the native enzyme (a reduced minus oxidized difference spectrum with maxima at 447 and 607 nm in the Soret and alpha regions, respectively). As judged from CO-reduced spectra this enzymatically digested, one subunit oxidase was found to contain an equal amount of cytochrome a and a_3. The enzymatic activity, using reduced cytochrome c as substrate, proceeded with equal affinity (apparent K_m of 0.5-1.0 µM) and V_{max} (the assays were carried out in Triton X-100) was one fifth (about 40 s^{-1}) of that found with the native enzyme (200s^{-1}).

BIO 139

2D NMR STUDIES OF THE DNA-BINDING DOMAIN 1-76 OF THE c2 REPRESSOR PROTEIN OF SALMONELLA PHAGE P22 AND A 16-BASE PAIR DNA FRAGMENT RELATED TO THE O_R1 BINDING SITE

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P22 c2 repressor was isolated from a strain of over-producing E. coli cells and the N-terminal fragment was recovered after chymotrypsin cleavage. Two-dimensional ^{1}H-NMR yielded the sequence-specific resonance assignments. Information from short proton-proton distances, observation of slowly exchanging amide protons, and scalar coupling constants was combined to determine the three-dimensional structure in solution using the distance geometry program DISMAN. Nitrocellulose-filter binding studies were used to assess the binding of the repressor fragment 1-76 to DNA fragments of variable length and sequence. A 16-base pair fragment of the operator region was then synthesized in mg-quantities. The binding constant of this DNA fragment with repressor 1-76 was determined by filter binding studies to be $4 \cdot 10^6$ $(mol/l)^{-1}$. NMR measurements gave an average lifetime of the complex of about 10 ms. Initial 2D NMR studies of the 1:1 protein-DNA complex will be presented.

BIO 140

CRYSTALLISATION OF LAMB PROTEIN FROM E. COLI OUTER MEMBRANE

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LamB protein, or maltoporin, forms a pore in the outer membrane of E. coli which facilitates the diffusion of maltose and higher maltodextrins. The native structure of the protein is a trimer which dissociates only at high temperatures or upon treatment with strongly denaturing agents. In the present work, maltoporin is isolated form E. coli K12 by extraction with octyl polyoxyethylene and subsequent chromatographic purification in presence of the same detergent. Crystallisation is achieved by the microdialysis method using polyethylene glycol as a precipitant. Single crystals can be grown from solutions of maltoporin in moderately high salt, neutral or slightly acidic pH and various detergents such as C_8E_5, β-octyl glucoside and mixtures of the two. In all cases investigated so far, the protein retains its native trimeric structure. The possibility of obtaining large single crystals is an important step towards elucidation of the three-dimensional structure of the integral membrane protein.

BIO 141

STRUCTURAL SIMILARITIES BETWEEN REACTION CENTER -AND ANTENNA POLYPEPTIDES OF PURPLE NON-SULFUR BACTERIA

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Our approach to understanding the role of pigment-protein interactions is to select and work out decisive structural features of antenna specific light-harvesting polypeptides (PP). The primary structures determined so far did provide considerable insight into pigment-protein binding. Besides the most significant structural feature, the overall conserved transmembrane histidine the antenna specific structural elements are clustered into groups of 2-4 amino acids containing 1-2 aromatic residues. A careful comparison (reversed alignement N/C) of the primary structures of the core antenna PPs α/β and the reaction center PPs L and M revealed: 9 out of 11 aromatic residues forming part of the special pair binding site (Michel et al. (1986) EMBO J. 5/10, 2445) are distributed in 5 distinct clusters which appear as structural elements in the core antenna PPs as well. On the basis of this study, considerable evidence is presented that in bacterial antenna -and reaction center complexes side chain aromaticity provides the major interaction site of the spectrally (absorption) equal but functionally different pigment types.

BIO 142

SEMISYNTHETIC SINGLE-SITE DIRECTED MODIFICATIONS OF CYTOCHROME *c*

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A new method of fragment condensation has recently been developed, combining enzymatic and chemical coupling methods (Rose, K. et al. Biochem. J. in press).

Cytochrome *c* can be cleaved at various sites, under specific conditions, to give several two-fragment non-covalent complexes. The C-terminus of an appropriate fragment is then activated by attaching an amino acid active ester by reverse enzyme proteolysis.

The activated C-terminus is brought into proximity to the N-terminus of the other fragment, since the two fragments form a non-covalent complex. Spontaneous coupling then occurs, under aqueous conditions.

Semisynthetic analogues of cytochrome *c* have thus been prepared in which single side chains, highly conserved in all mitochondrial cytochromes *c*, have been replaced by other functional groups. In this way, the role of these conserved residues in the structure-function relationship can be investigated.

BIO 143

REDUCTION OF BILIRUBIN BY BACTERIAL ENZYMES

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Bilirubin is enzymatically reduced to a mixture of urobilinogens by the action of intestinal bacteria. However, the enzymes have never been isolated so far. As a preliminary to the possible isolation of the reducing enzymes, incubation conditions were studied to observe conversion of bilirubin by broken cells preparations. Protease inhibitors were included to prevent autolysis. Clostridium ramosum was cultured anaerobically, at 37°C, in a thioglycolate medium without glucose. After centrifugation,the microorganisms were suspended in the incubation medium, sonicated, bilirubin was added and the mixture was incubated aerobically at 37°C. The formation of urobilins was observed by the increase in absorbance at 487 nm. Results: at pH 7.6 and 8.0, the solution containing bilirubin and the sonicate showed an increase in absorbance at 487 nm; the changes were greater at pH 7.6; they were linear over the first 3 minutes; no increase was noted at pH 8.6; addition of EDTA enhanced the conversion; no effect of NADH addition was observed; a non-enzymatic reduction was observed in controls incubated with boiled sonicate(less than 12% of the activity).

BIO 144

STRUCTURE AND FUNCTION OF L-LACTATE DEHYDROGENASES (LDH) FROM THERMOPHILIC AND MESOPHILIC BACTERIA

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Protein engineering techniques have been used to study structure-function relationships of bacterial LDHs. Wildtype and hybrid enzymes as well as single replacement mutants containning moieties of enzymes from thermophilic (B. stearothermophilus, B. caldolyticus) and mesophilic bacilli (B. megaterium) were compared. Overall, thermostability was enhanced most significantly by the amino acid exchanges Ala → Val and Thr/Ser → Ala; the effects of substitutions on thermostability were non-additive. To study structural features responsible for the function of the activator fructose-1,6-bisphosphate (FDP), we prepared hybrid-mutants from segments of the LDHs (97% similarity) from B. stearothermophilus (activated by FDP) and B. caldolyticus (not activated). Five amino acids in the middle part of the sequence were essential for activation. A hybrid-LDH comprising parts of the B. stearothermophilus and the B. megaterium enzymes (both FDP-activated) was not activated, indicating that activation cannot be attributed to a unique set of amino acids.

BIO 145

INHIBITION OF PURIFIED CHOLINE-O-ACETYLTRANSFERASE (CHAT) FROM THE ELECTRIC ORGAN OF TORPEDO MARMORATA BY DERIVATIVES OF (2-BENZOYLETHYL)TRIMETHYLAMMONIUM CHLORIDE (BETA)

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BETA is known as a selective and stable inhibitor of ChAT which catalyzes the synthesis of acetylcholine. The inhibition is slowly reversible and noncompetitive with respect to both substrates of ChAT, acetyl-CoA and choline. We have investigated the inhibitory effects of a series of derivatives of BETA on the activity of ChAT isolated from the electric organ of Torpedo marmorata. The enzyme was purified by a procedure involving four steps, with affinity chromatography on agarose-hexane-coenzyme A as the last step. The possible targets for structural changes in BETA are: the benzoyl portion of the molecule and the quaternary ammonium group. Replacement of the quaternary ammonium group by a ternary ammonium group resulted in the loss of inhibitory activity by several orders of magnitude. Substitution at the phenyl ring on position 3 or 4 with a hydroxy-, nitro-, azido- or amino-group resulted in compounds some of which showing a stronger inhibitory activity than BETA.

BIO 146

DISTRIBUTION AND PHARMACOKINETICS OF THE rDNA SECRETORY LEUKOCYTE PROTEINASE INHIBITOR (SLPI)

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SLPI is a human proteinase inhibitor of Mr 11'700. It is a potent inhibitor of leukocytic elastase and cathepsin G. SLPI may be therapeutically useful in diseases where these enzymes are involved e.g. in pancreatitis, shock and emphysema. Distribution of SLPI was determined in rats after i.v. bolus injection of 2mg/kg ^{35}S-SLPI. Five minutes after injection kidneys, blood and liver contained 30, 20, and 10%, respectively, of the injected radioactivity. Low amounts were detected in adipose tissue, muscle and brain. After injection of cold SLPI, its plasma concentration was determined by ELISA. The concentration versus time (half logarithmic plot) shows two linear segments, representing the distribution and the elimination phase with half time values of 6-8 and 38-42 minutes respectively. These values are comparable with those of Eglin c, an inhibitor from the leech.

BIO 147

TWO L-GLUTAMATE DEHYDROGENASES FROM THE ALGAL SYMBIONTS (SYMBIODINIUM MICROADRIATICUM) OF CORALS

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The dinoflagellate endosymbionts ("zooxanthellae") of corals are thought to be responsible for primary ammonium assimilation in the intact association, scavenging ammonium released by host catabolic processes and taken up from the environment; the predominant route of ammonium assimilation in zooxanthellae is not at present clear. In view of its low affinity for substrate (K_m for NH_4^+ in the range 1 - 10 mM), L-glutamate dehydrogenase (GDH) is not thought to play a significant role in ammonium assimilation in plants, however some eukaryotic algae may be exceptions to this rule. We have purified and characterized two GDHs from zooxanthellae. An NADPH-GDH displayed biphasic kinetics with ammonium as the variable substrate, the apparent K_m at low [NH_4^+] being below 1 mM, and the enzyme had very low deamination activity; these results suggest a role in ammonium assimilation. A second NADH-specific GDH was also identified, and presumeably functions in glutamate oxidation in vivo.

BIO 148

31-P NMR MAGNETIZATION TRANSFER IN THE RAT BRAIN: EFFECTS OF BARBITURATES AND CALCIUM ANTAGONISTS

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The rate constant (k) for the forward creatine kinase reaction (PCr+ADP+H$^+$-> ATP+Cr) was measured in the living rat brain by the NMR technique of magnetization transfer using a surface coil. Using an average T1(PCr) of 2.0±0.3 sec, the following k-values (mean±SE) have been found:

	Halothane	Pentothal	PN 200-110		
mg/kg ip	(2.5%)	40	0.5	2.5	5.0
k [sec^{-1}]	0.68 ±.03	0.51 ±.03	0.61 ±.04	0.53 ±.04	0.55 ±.04

Assuming that brain energy transfer via the creatine kinase reaction is closely coupled to energy production, as has been shown for the heart (Bittl & Ingwall, JBC 260 3512 (1985)), and since steady-state ATP levels remain constant, these results suggest that the calcium antagonist PN 200-110 reduces brain energy consumption. This effect of PN may be relevant for its cytoprotective activity, since reduction of infarct size in a rat stroke model has been found at similar doses (Stroke 17 1228 (1986)).

BIO 149

CONTENTS OF WATER-SOLUBLE SCAVENGERS IN NEOPLASTIC AND NON-NEOPLASTIC HUMAN BREAST TISSUE

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The scavengers ascorbic acid (Asc), cysteine (Cys), glutathione (GSH) and uric acid (UA) were determined in 50 protein-free extracts of breast tissue (neoplastic and non-neoplastic from 25 patients), using a reversed phase HPLC method and electrochemical detection. DNA content was also measured. Epithelium and fat as percent of section area were estimated in haematoxylin-eosin stained sections from tissue adjacent to the sample. Scavenger values in nmol/g DNA, a parameter related to content per cell, were higher in neoplastic tissue for Asc (24/25 cases, average +129%), for GSH (22/25, +93%) and for Cys (18/25, +31%) and lower for UA (24/25, -80%). As amounts of Asc and GSH correlated with percent epithelium, the increases in these two substances in neoplastic tissue were very probably located in these cells. It is known that increased GSH protects cells against certain cytostatics in tissue culture. For in vivo treatment of breast cancer with these drugs, the presence of increased Asc besides GSH could be of importance.

BIO 150

REGULATION OF GLUCOSE TRANSPORT AND TRANSPORTER SYSTEM IN RAT HEART : EFFECT OF INSULIN, WORKLOAD AND GLUCOSE PER SE.

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The regulation of D-glucose transport by perfused hearts of normal rats was studied, as well as glucose transporters, GT (labelled cytochalasin B binding). Insulin or increased workload stimulated glucose transport 7 to 8-fold, glucose 4-fold. The three stimuli favored translocation of transporters from microsomal to plasma membranes (PM). Insulin altered Kd values of PM-GT and increased Hill coefficient (positive cooperativity amongst PM-GT). Workload increased only Hill coefficients, glucose altered only Kd values. To conclude : insulin, workload or glucose stimulate glucose transport by favoring the translocation of GT and by changing, albeit differently, the functional properties of the PM-GT.

BIO 151

RESTORATION OF INSULIN RESPONSE IN BROWN ADIPOSE TISSUE OF GENETICALLY OBESE fa/fa RATS BY A ß ADRENERGIC AGONIST.

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Brown adipose tissue (BAT) of obese fa/fa rats has defects in insulin-induced glucose transport, and reduced catecholamine turnover. To investigate a role of decreased catecholamine turnover on the impaired insulin response, fa/fa rats were given a ß adrenergic agonist, RO-16-8714, that was administered twice, 24h apart. In anesthetized fa/fa rats previously treated with RO, a marked increase in glucose utilization was measured in BAT (basal conditions). Glucose utilization was further stimulated in BAT after a hyperinsulinemic-euglycemic clamp. The data indicate that RO administration to fa/fa rats restores both basal and insulin-induced glucose uptake by obese rat BAT, to normal.

BIO 152

TASTE-INDUCED CHANGES IN PLASMA AND GLUCOSE TURNOVER IN LEAN AND GENETICALLY OBESE FA/FA RATS.

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Freely-moving lean and obese rats were trained to drink 1 ml of sodium saccharin (0.15%). At 1 min post-stimulus, there was an increase in cephalic insulin release in lean and obese rats whose amplitude was higher in the latter than in the former group. Saccharin produced increases in hepatic glucose production (HGP) and in the rate of glucose disappearance (Rd) in lean and obese rats when compared to basal values. Saccharin-induced changes were higher in the obese group. Thus, saccharin (through taste) appears to elicit parasympathetic (insulin release) and sympathetic (HGP increase) reflexes in both groups. All taste-induced changes are exaggerated in obese animals.

BIO 153

EFFECT OF INSULIN ON GLUCOSE TRANSPORT AND TRANSPORTERS IN BROWN ADIPOSE TISSUE (BAT) OF LEAN AND GENETICALLY OBESE FA/FA RATS.

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Laboratoires de Recherches Métaboliques, Geneva, Switzerland

Glucose transporters (GT) in plasma (PM) and microsomal membranes (MM) were studied in BAT of lean and obese rats after a hyperinsulinemic-euglycemic clamp. In lean rat BAT, insulin produced a 40-fold increase in glucose utilization that was associated with : a translocation of GT from MM to PM, without change in total number GT ; an increase in Hill coefficient of PM-GT (from 1.1 to 2.5), i.e occurence of a positive cooperativity ; a decrease in the Kd values of PM-GT (increased affinity of PM-GT). In the obese rats the smaller increase in glucose utilization was associated with normal translocation of GT, but without change in the properties of PM-GT. Thus, both glucose transporter translocation and activation are necessary for a full stimulatory effect of insulin.

BIO 154

PURIFICATION AND CHARACTERIZATION OF THE SUBUNITS FORMING THE MEMBRANE PART (F_o) OF THE ATP SYNTHASE (F_1F_o) OF E.coli

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Three different subunits, a, b and c, the latter known as the DCCD-binding proteolipid, constitute the proton-conducting, membrane-integrated moiety (F_o) of the ATP synthase complex in E. coli, to which the catalytic F_1 moiety is attached. We have developed a new method for the preparation of the individual F_o subunits in a pure, active and fairly stable form. The method involves the complete solubilization of F_o as individual subunits with a zwitterionic detergent and high resolution chromatography (FPLC). Advantages of this method are high recoveries and no need for chaotropic agents to keep the F_o subunits dissociated. By reconstitution into phospholipid vesicles the function and exact stoichiometry of the individual subunits in the active F_o complex are being investigated. Additionally, work is in progress to complement the reconstitution experiments with specific chemical modification to address questions about the localization and function of specific amino acids in the active F_o complex.

BIO 155

CONTROL BY CA^{2+} OF PYRUVATE METABOLISM IN RAT LIVER MITOCHONDRIA

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Treatment of whole liver or of liver cells with Ca-mobilizing hormones can modify the function of subsequently isolated mitochondria. Disagreement exists on the possible physiological role of excess Ca taken up by the mitochondria, especially with regard to pyruvate metabolism. Part of these discrepancies can clearly be attributed to differing experimental designs as shown in the present study. Mitochondria containing low Ca-contents were exposed either to Ca-pulse additions (0-50 µM, maximally 25 nmoles/mg protein) or to Ca-EGTA buffers at 5 mM EGTA and free Ca-concentrations in the 0-50 µM range. Pyruvate carboxylation is insensitive towards pulse additions of Ca up to 50 µM, whereas strong inhibition was observed in incubations with Ca-EGTA buffers exceeding 10 µM free Ca. The stimulation of pyruvate decarboxylation is also less sensitive towards Ca-pulses than towards Ca-EGTA buffers even though mitochondrial Ca-contents were higher after Ca-pulses. These results indicate a novel effect of the Ca-buffering system.

BIO 156

A SPIN LABEL ELECTRON SPIN RESONANCE STUDY OF THE BINDING OF MITOCHONDRIAL CREATINE KINASE TO CARDIOLIPIN

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The binding of the mitochondrial creatine kinase to cardiolipin has been studied via the perturbation of the mobility of spin labelledcardiolipin, using electron spin resonance spectroscopy. In the presence of creatine kinase (1:1 w/w), the ESR spectra of cardiolipin labelled in a single acyl chain indicate a restriction of motion both at the C-5 and C-14 positions of the lipid chain. The effect of the protein on the chain mobility is consistent with a surface binding of the protein; no positive evidence was obtained for penetration of the protein into the hydrophobic membrane.

BIO 157

BINDING OF ADP TO RAT LIVER CYTOSOLIC PROTEINS

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In a cytosolic extract from rat liver, the number and the concentration of ADP binding sites as well as their dissociation constants were determined using the rate of dialysis technique. Interfering cytosolic adenylate kinase was extracted from the cytosol by affinity chromatography on Ap_5A-Sepharose. Remaining traces of adenylate kinase activity were inhibited with (+)-catechin. Binding of ADP to cytosolic proteins was increased by polyethylene glycol and decreased by EDTA. The effect of 10^{-4}M EDTA could be reversed by addition of equimolar concentrations of Mn^{2+} or Mg^{2+}. In presence of 5% polyethylene glycol, added to increase local protein concentration, two binding sites for ADP were observed with K_D values of 1.9×10^{-6}M (site I) and 10.8×10^{-6}M (site II). The concentration of these binding sites, when extrapolated to cellular protein concentrations, were 30×10^{-6}M (site I) and 114×10^{-6}M (site II). It is concluded that about 50% of total cytosolic ADP is bound to proteins and that the ratio of free ATP/free ADP is about twice that of total ATP/total ADP.

BIO 158

INHIBITION AND POTENTIAL IDENTIFICATION OF THE Na^+/P_i-COTRANSPORTER BY/WITH N-ACETYL-IMIDAZOLE

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In order to identify proteins involved in renal Na^+/P_i-cotransport, the group (tyrosine) specific reagent N-acetyl-imidazole (NAI) has been used. In renal brush border membrane vesicles (BBMV) P_i-transport is inhibited 80% by 30 mM NAI and in OK-cells $^{32}P_i$-transport is inhibited 70% by 10 mM NAI. NAI inhibition could be protected (by 25% in BBMV and by 80% in OK-cells) by the presence of 5 to 10 mM P_i. Protection was specific, since the inhibition of other Na^+-dependent transport processes by NAI was not affected by the presence of P_i.
By an acetylation of the BBMV and OK-cell proteins with [^{3}H]-NAI either in the absence or in the presence of P_i, some proteins could be identified in which P_i-protected acetylation was observed and which therefore can be regarded as potential canditates for the renal Na^+/P_i-cotransport system.

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BIO 159

INSERTION OF A MULTI-SPANNING MEMBRANE PROTEIN

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To study how multi-spanning membrane proteins are inserted into the endoplasmic reticulum, we constructed artificial membrane proteins on the cDNA level by repeating the internal signal-anchor domain of the asialoglycoprotein receptor H1 up to four times. Upon in vitro translation in the presence of microsomes these polypeptides are indeed inserted as multi-spanning membrane proteins: the first hydrophobic domain functions as a signal sequence, the second as a stop-tranfer sequence and the third initiates a second translocation process, halted again by the fourth. We were able to demonstrate that insertion occurs cotranslationally and seqentially. By replacing the third hydrophobic domain by a mutant domain unable to interact with signal recognition particle (SRP), we could show that the second insertion step does not require SRP and that only the first hydrophobic domain needs to be a functional signal sequence.

BIO 160

INCORPORATION AND LATERAL DIFFUSION OF A SYNTHETIC MITOCHONDRIAL SIGNAL PEPTIDE IN MODEL MEMBRANES

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A fluorescent synthetic signal peptide corresponding to the presequence of mitochondrial cytochrome c oxidase subunit IV was prepared by labelling with iodoacetoxyethyl-NBD at cysteine-19. This peptide (NBD-cox IV-25) incorporated into phospholipid monolayers with similar affinity as the unlabelled peptide. Titration experiments with lipid vesicles of different composition revealed that NBD-coxIV-25 binds and inserts into bilayer model membranes by a similar, charge dependent mechanism as into monolayers. The peptide lateral diffusion coefficient in reconstituted membranes was relatively low ($\sim 1 \mu m^2/s$), perhaps indicating the formation of oligomers.

BIO 161

EFFECT OF TRYPSIN ON THE PERMEABILITY AND STRUCTURE OF Na,K-ATPase-LIPOSOMES

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Cholate-dialysed liposomes containing dispersed, randomly reconstituted Na,K-ATPase molecules were used to investigate the effect of trypsin on the permeability and structure of the pump. It is well known that trypsin attacks the intracellular part of the transmembrane alpha-subunit. Thus, trypsin was not expected to affect the transport-activity catalyzed by the right-side-out oriented pump population. In fact, low trypsin concentrations had no effect on the transport activity of the right-side-out oriented pump-population during the linear Rb-uptake phase. However, when the trypsin/Na,K-ATPase ratio was increased to 1:1 (w/w), the Rb-content of the liposomes decreased by 80 to 90% within 15 min at 25°C. The results indicate that the intracellular component of the Na,K-ATPase molecule contains a trypsin-sensitive barrier. The structural alterations are analysed by gel-electrophoresis of the trypsin-treated Na,K-ATPase-liposomes. SNSF 3.502.0.86.

BIO 162

LIPIDABSORPTION IN BRUSH BORDER MEMBRANE

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When brush border membrane vesicles prepared from rabbit small intestine are either incubated at 4°C (pH7.1) or digested with papain, proteins are released into the supernatant. The protein(s) is (are) responsible for the incorporation of lipid (phosphatidylcholine, cholesterylester) into brush border membrane. It (they) also catalyze(s) the exchange of phospholipids from small unilamellar lipid vesicles and micelles to brush border vesicles and also between different populations of unilamellar lipid vesicles. Furthermore, it can be demonstrated that the protein(s) has (have) fusogenic activities in phospholipid vesicles. The activities described above can be attributed to integral brush border membrane protein(s) and are not due to contamination of the brush border membrane by basolateral membranes or endoplasmatic reticulum.

BIO 163

INTRACELLULAR SORTING OF THE RABBIT POLYMERIC IMMUNOGLOBULIN RECEPTOR (pIgR) IN A RECONSTITUTED SYSTEM

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Mammary cells stably transfected with pIgR cDNA were grown on Nucleopore filters and the processing of pIgR was analyzed by pulse-chase experiments and selective cell surface trypsinization or radioiodination. The pIgR is routed to the basolateral membrane where it binds its ligand. It is phosphorylated during intracellular transport. At the apical membrane where the receptor is cleaved into secretory component intact receptor is still found, indicating that cleavage is a rate limiting step. At the basolateral cell surface the receptor appears further phosphorylated and oligomerized. These forms are only recognized by the anti-tail antibodies and are not detected at the apical membrane. Conformational change of the receptor, as detected with different antibodies, is concomitant with phosphorylation. It may be that phosphorylation acts as a sorting signal.

BIO 164

PHOTOAFFINITY LABELING OF THE γ-BUTYROBETAINE BINDING PROTEIN FROM AN AGROBACTERIUM SP.

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The photoaffinity reagent S-(p-azidophenacyl)thiocarnitine (PAP-TC) has been synthesized according to Mauro et al., 1986. This compound, originally designed for the study of carnitine acetyltransferase, was tested on the Agrobacterium γ-butyrobetaine transport system. PAP-TC is an active-site-directed reagent for the transport system, since it showed a competitive inhibition (K_i=70 uM) of γ-butyrobetaine transport. UV irradiation of periplasmic proteins in the presence of PAP-TC (^{14}C) resulted in the irreversible labeling of the γ-butyrobetaine binding protein. The addition of 3 mM γ-butyrobetaine in the mixture,significantly decreased the incorporation of the reagent, showing that this compound binds specifically to the active site of the binding protein.

BIO 165

PURIFICATION AND CHARACTERIZATION OF RECONSTITUTIVELY ACTIVE MITOCHONDRIAL ANION-CARRIERS FROM BOVINE HEART AND LIVER

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Transport competent 2-oxoglutarate-carrier was purified to near homogeneity (apparent molecular weigth of 31500) from heart and liver mitochondria. In both cases, the 2-oxoglutarate-carrier was extracted from mitochondria with 3% Triton X-114 in the presence of 2 mg/ml cardiolipin and purified by chromatography on hydroxylapatite,celite and organomercurial agarose (Affigel 501, Biorad). Reconstitution of the purified heart mitochondrial carrier was obtained by removal of Triton X-114 using Amberlite XAD-2 beads. It allowed to measure the specific activity of the purified carrier (3 μmol/min/mg protein) which increased 20 fold by passing the hydroxylapatite eluate through celite and Affigel 501. Inhibition (≥ 95%) was obtained by eosin-5-maleimide,p-chloromercuribenzoate and mersalyl but not by N-ethylmaleimide. Cholesterol (5% w/w), included in the liposomes, increased the transport activity by a factor 2. By using organomercurial agarose gels with different spacer-lengths the dicarboxylate and tricarboxylate carrier from liver mitochondria were also partially purified in an active form.

BIO 166

USE OF AMILORIDE-SENSITIVE NA+ CHANNEL SUBUNIT ANTIBODIES AND PUTATIVE cDNA TO EXAMINE mRNA LEVELS AND CELL SURFACE EXPRESSION OF THE CHANNEL.

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An amiloride-sensitive Na channel is localized to the apical plasma membrane of high resistance epithelia. To characterize this channel, we utilized a cDNA clone obtained from an A6 expression library. Antibodies raised against expressed protein from this cDNA clone specifically bind the Na channel purified from A6 cells. The cDNA hybridizes to 5.2, 5.0, and 2.9 kb mRNA from cells grown on collagen coated filters. The amount of specific mRNA was substantially reduced when cells were grown on plastic dishes. Cell surface expression of the channel was examined in cells grown on filters or on plastic, by radioiodination of the apical plasma membrane followed by immunoprecipitation. A substantial increase of apical cell surface expression of the channel was found when cells were grown on filters, compared to cells grown on plastic. In conclusion, anti-channel antibodies have been characterized and used to study its cell surface expression. Final proof that the cDNA contains partial sequence of the channel will require full length cDNA and reconstitution of a functional Na channel.

BIO 167

Food deprivation and restoration at dawn induces a disturbed circadian feeding rhythm.

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Circadian rhythms of food intake were continuously registered with a computerised food hopper system in 10 male Wistar rats (450-500g) before and after 3 days food deprivation beginning either at light onset or at light offset. In order to reduce "masking" effects of light itself, the rats were entrained to a skeleton 12:12 photoperiod with 1 hour light pulses. Food restoration at the beginning of the light, but not of the dark phase induced a rhythm disturbance with increased feeding occurring in the early light phase. This effect lasted 4-7 days and gradually disappeared. Food restoration at dawn increased the rebound 24-hour food intake to a greater extent and for a longer time (reaching previous body weight earlier) than when given at dusk. This "damped oscillation" induced at an antiphase to the normal feeding rhythm, can be related to the feeding-entrainable oscillator and to the circadian cycle of lipolysis and lipogenesis, both of which are mediated by the medial hypothalamus.

BIO 168

IMMUNOAFFINITY ISOLATION AND INITIAL CHARACTERISATION OF APOPROTEIN E-CONTAINING SUBCLASSES OF HIGH DENSITY LIPOPROTEINS.

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Apolipoprotein E (apoE) is a receptor ligand found in several lipoprotein classes.The apoE-containing high density lipoprotein (HDL) subclasses may be of physiological importance, particularly in the "reverse cholesterol transport" pathway. The present study investigated the apoE-containing subclasses of HDL_2 and HDL_3 separated from normal human plasma by density ultracentrifugation. We have used an immunoaffinity column prepared with a monoclonal anti-apoE antibody to separate HDL into HDL-with apoE and HDL-without apoE. The apolipoprotein composition of the fractions was examined by SDS-PAGE. No trace of apoE was found in HDL_2- and HDL_3-non-bound fractions indicating that the mAb recognized all HDL-bound forms of apoE. HDL_2-with apoE and HDL_3-with apoE contained as major bands, apoE and apoA-I, and traces of apoA-II and apoC. However, a major difference between the two fractions was the E:A-I molar ratio (for HDL_2:2.65; for HDL_3: 0.39). No apoE was detected unassociated with apoA-I in HDL_2 and HDL_3.

BIO 169

IN VITRO BIOSYNTHESIS OF INOSITOL CONTAINING LIPIDS IN TRYPANOSOMATIDAE.

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Particulate fraction prepared from Trypanosoma brucei brucei homogenates obtained by moderate sonication, is able to catalyse the incorporation of free myo-inositol into lipids, mainly phosphatidylinositol (PI). The enzyme needs Mn^{++} ions, while Mg^{++} is ineffective. CDP-diglyceride (dipalmitate) exogenously added, failed to show any stimulation effect, suggesting the enzymatic reaction beeing an exchange. Phospholipids (PS, PC, PA, PA + CTP), were tested as potential precursors and were found ineffective.
A K_M of 30 uM was found for inositol in the presence of 4.8 mM Mn^{++}. The reaction was nearly linear during the first 40 min. of reaction and Mn^{++} stimulation was non hyperbolic in relation with its concentration.
In contrast, similarly prepared homogenates of Leishmania major showed reproducible stimulation by added CP-diglyceride. The reaction was slightly stimulated by Mg compared to the effect of Mn^{++} at the same concentration.

BIO 170

STRUCTURE DETERMINATION BY NMR OF A MEMBRANE LIPID (LIPID B) OF OCHROMONAS DANICA (CHRYSOPHYCEA)

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Lipid B was detected in the unicellular alga Ochromonas danica in 1970 (1). The structure of this major membrane component, however, has not been elucidated so far. The lipid which is free of both phosphorous and carbohydrate, was isolated from cells by medium pressure liquid chromatography (MPLC) and thin layer chromatography (TLC). Although this component is unstable, the following structural elements could be assessed by ^{1}H and ^{13}C NMR: Two fatty acids are esterified to a glycerol which is linked by an ether bond to the polar group. The latter contains a N,N,N-trimethyl group which is very easily split off as trimethylamine. In its structure, lipid B is very likely related to the already known diacylglyceryl-(N,N,N-trimethyl)homoserine (DGTS) which is also present in this alga, as well as in many other cryptogamic plants.

(1) B.W. Nichols (1970). In: Phytochemical Phylogeny (J.B. Harborne, ed.) pp. 105-117. Academic Press, London and New York

BIO 171

QUANTIFICATION AND CHARACTERIZATION OF HUMAN APOLIPOPROTEIN B WITH MONOCLONAL ANTIBODIES

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Human low density lipoprotein (LDL) is the major cholesterol carrier in the bloodstream. It contains one large protein molecule, apolipoprotein B (apo B). Various evidence has established that an increased concentration of apo B in plasma is associated with an increased risk for coronary artery disease. We have selected 4 monoclonal antibodies (MAb) that recognize different epitopes on apo B. Two of them, namely A1G10 and F5D5, are directed against common epitopes on apo B. Their suitability for quantifying apo B in plasma was investigated in 3 different ELISA-systems: a double antibody sandwich assay, an inhibition of binding of biotinylated MAb to coated standard-LDL and a competition in binding to coated MAb between biotinylated standard-LDL and apo B in plasma. MAb D2E1 and H11G3 detect epitopes c and d of a genetic polymorphism of apo B - the so-called Ag-system. These MAb were used for phenotyping apo B in plasma.

BIO 172

IN VIVO ^{31}NMR PHOSPHOMONOESTER MEASUREMENTS CAN BE USED TO FOLLOW BRAIN DEVELOPMENT

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By using nuclear magnetic resonance (NMR) spectroscopy it is possible to measure in vivo and noninvasively brain phosphate containing compounds, such as phosphomonoesters (PME). With analytical in vitro ^{31}P NMR we showed that the PME peak of the rat brain contains phosphorylethanolamine (PE) and to a minor extent phosphorylcholine. PE is known to be a precursor of the phospholipid phosphatidylethanolamine (PhE), an important compound in brain membranes. In order to correlate in vivo NMR data with classical biochemical data we determined by ^{31}P NMR the age dependent changes of PME, observing the same animals during their development from 2 to 56 days. In parallel, PE and PhE were measured biochemically. We found a clear correlation between the age dependent decreases of PME and PE, and the aequivalent increase of PhE in the period of rapid myelinogenesis. These results indicate that in vivo ^{31}P PME measurements are useful to follow normal brain development.

BIO 173

DIFFERENT LEUKOTRIENE (LT) FORMATION BY HORSE (h) AND BOVINE (b) EOSINOPHILS

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H-eosinophils (>98% pure), stimulated by low concentrations (<4 µM) of the Ca-ionophore A23187, generate predominantly sulfidopeptide LTs (LTC_4, LTD_4 and their 11-t isomers). At higher concentrations of A23187 LTs of the B_4-type are also produced. B-eosinophils (>95% pure) generate at low A23187 concentrations only small amounts of LTs of the B_4-type and no sulfidopeptide LTs. With increasing A23187 concentrations, the LT-formation of the B_4-type increases but no further leukotrienes are detected. However, the B_4 pattern is different to the B_4 pattern generated by h-cells. H-eosinophils, stimulated by 20 µM arachidonic acid (AA), generate predominantly sulfidopeptide LTs. H-eosinophils, stimulated by 80 µM AA also produce high amounts of LTs of the B_4-type. By increasing the AA concentrations, in b-cell incubations, an increased LT-formation of the B_4-type is detected, but no LTs of the sulfidopeptide-type are formed. The HPLC ($A_{237\ nm}$) and the radiochromatogram of extracts of stimulated h-cells reveal one peak with the retention time of the 5-HETE; for the extracts of b-cells, two peaks were detected. A major peak which is probably a 15-HETE and a minor peak with the retention time of 5-HETE were found.

BIO 174

ISOLATION AND QUANTITATIVE DETERMINATION OF THE CARBOHYDRATE STRUCTURES OF HUMAN C1s, A SUBCOMPONENT OF THE COMPLEMENT SYSTEM.

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C1s, isolated from serum with chromatographic methods was activated with C1r. The A- and B-chains of reduced and alkylated C1s were separated by ion exchange chromatography on DEAE-Sephacel. The A-chain gave a carbohydrate positive reaction and, according to amino acid sequencing, is expected to carry two N-glycans, while the B-chain is apparently devoid of carbohydrate. Hexosamines, hexoses and sialic acids were liberated by different hydrolysis conditions and quantitated by the ninhydrin reaction or by reversed phase HPLC with pre-column-derivatization. The contents of Sia, Gal, Man and GlcNAc (GalNAc) were 1.1, 1.2, 1.3 and 2.4 (0.5) %, respectively. The carbohydrate composition (mol/mol protein) is therefore : Sia 2.9, Gal 4.9, Man 5.8 and GlcNAc 7.5 (GalNAc 1.5). These results may be indicative for the presence of two di-antennary N-acetyllactosamine type glycans, possibly differing from one another in the extend of sialylation.

BIO 175

CLONING OF THE α-AMYLASE GENE OF SCHWANNIOMYCES CASTELLII AND SECRETION OF ITS GENE PRODUCT IN DIFFERENT YEAST GENERA

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The α-amylase gene of the yeast SCHWANNIOMYCES CASTELLII was cloned by functional complementation in S. CEREVISIAE.DNA sequencing revealed an open reading frame of 1536 bp.The gene is expressed and the active gene product is secreted in S. CEREVISIAE,KLUYVEROMYCES LACTIS , SCHIZOSACCHAROMYCES POMBE and HANSENULA POLYMORPHA .For expression and secretion in HANSENULA POLYMORPHA the SCHWANNIOMYCES CASTELLII promotor was exchanged by an inducible methanol oxidase promotor of HANSENULA POLYMORPHA. Further details can be discussed during the poster session.

Plant Physiology (PLA)

PLA 176

OZONE IN AMBIENT AIR DISTURBS CARBON ASSIMILATION IN WHEAT

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Ozone is known to reduce carbon assimilation and yield of wheat. In order to investigate the mechanisms of this effect on ribulosebisphosphate-carboxylase (RuBPCO) and photosynthesis, wheat was grown in open-top chambers with charcoal-filtered, unfiltered and ozone-enriched air (15, 30, 70 and 100 ppb O_3; 8h/day saisonal mean). CO_2 uptake of flag leaves was measured before, during and after anthesis. Leaf sections were sampled after freezing for enzymatic analysis. With increased O_3, the decrease in photosynthesis was associated with a decrease in chlorophyll and activatable RuBPCO. Parallel to dark respiration, the CO_2 compensation concentrations in 21% O_2 and in 2% O_2 increased with increasing O_3, thus indicating negativ effects on assimilates in ozone-stressed tissues. The activation of RuBPCO was enhanced at higher O_3 levels. Increasing O_3 affected also the "assimilation power" which was characterized by increased ratios of ATP/ADP and Triose-P/PGA. The relative increase in triose-P in ozone-stressed leaves suggests that catalysed steps other than only carboxylation of RuBP were affected in the Calvin cycle. This could lead to an accumulation of starch in the chloroplasts and to premture leaf senescence.

PLA 177

DIRECT CONVERSION FROM ROOT TO SHOOT MERISTEM IN ARABIDOPSIS THALIANA

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In an auxin containing medium callus from leaf explants regenerates root primordia which grow out into roots in a hormonfree medium. Shoot inducing medium converts the root meristem into a shoot meristem which develops via a green globular structure to a complete shoot system.

PLA 178

MICROCULTURE AND ELECTROFUSION OF DEFINED PAIRS OF BRASSICA PROTOPLASTS:FROM SINGLE CELLS TO PLANTS

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The improved microculture system developed for Nicotiana tabacum protoplasts was adapted to the individual culture of rapeseed protoplasts in microdroplets of fully synthetic and unconditioned medium. This single cell culture system allowed microcallus formation frequencies of up to 50% from the selected protoplasts as well as the establishment of cell clones with morphogenetic potential. Based on this microculture technique the analysis of cell to cell interactions at the single cell level between different types of protoplasts was possible. The possibility of manipulating the extranuclear genetic material of plant cells promoted studies on plant cell reconstruction using subprotoplasts such as karyoplasts, microinjected karyoplasts and cytoplasts as one or both of the parents.High efficiency electrofusion of such defined pairs of protoplasts and subprotoplasts of B. napus is presented as well as the microculture of the fusion products leading to callus formation and regeneration of plants. Moreover this microculture system proved useful for individually selected protoplasts of the moss Funaria hygrometrica as well as for animal cells.

PLA 179

MICROINJECTIONJECTION TECHNIQUE:A USEFUL TOOL FOR PLANT ENGINEERING.

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Progress in the transformation of agriculturally important plants has been restricted by the limitations of presently available gene-transfer systems.Routinely plant transformation is achieved by Agrobacterium tumifaciens Ti-plasmid, which is limited by the host range of the bacterium, and by direct gene transfer, which excludes all plant species lacking a plant regeneration system. Therefore novel techniques are needed to extend gene transfer to other plant species,particularly cereals.As an alternative strategy we developed an efficient transformation technique based on intranuclear microinjection of DNA into plant protoplasts and subprotoplasts followed by single cell culture of manipulated cells. The efficiency of transformation in this system has been reported up to 60%. For plants lacking the regeneration capacity, the novel technique of microinjection into microspore-derived embryoids has been developed. These multicellular strutures have a high competence for plant regeneration through embryogenesis.Transformation has been proved by enzyme assay of the introduced gene and by Southern analysis.

PLA 180

DIRECT EMBRYOGENESIS FROM POLLEN OF CEREALS

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Immature pollen embryogenesis and subsequent plant regeneration of the major cereals including barley, maize, rice and wheat have been established in culture. Earlier work in cereal microspore culture revealed a poor regeneration efficiency mostly through a callus phase and high % albino plant formation. Our present results clearly show a very promising system of high frequency green plant formation through a direct embryogenesis avoiding a callus phase. Certain stress conditions particularly cold treatment improved the frequency of microspore divisions and green plant formation. Media containing high amounts of reduced nitrogen and Ficoll 400 (20 % w/v) favoured direct embryogenesis. In optimised conditions, plant regeneration in wheat took 28 days from the start of the culture, significantly shorter than that obtained for any other cereal system. This system should now provide the opportunity to improve cereal breeding programmes and vectorless gene transfer by micoinjection to potential microspore derived embryoids.

PLA 181

EFFECT OF CATIONS ON PHYTOALEXIN ACCUMULATION AND SECRETION BY SOYBEAN ROOTS

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Roots of soybean cultivars Harosoy and Harosoy 63 infected with zoospores of *Phytophthora megasp.* or induced with 1 mM $AgNO_3$ synthesize a variety of secondary products including the isoflavonoid phytoalexin glyceollin. We now present evidence that roots submerged in water release 50-60 % of the glyceollin into the medium. Two additional, more polar substances which can be detected already 4 h after induction are only found in the medium. They inhibit fungal growth to the same extent as glyceollin and may, therefore, be considered as phytoalexins. Addition of Ca (0,5-10 mM) reduces total synthesis of phytoalexins. This involves primarily the released compounds while those in the root tissue remain at approx. the same level. Conversely, 0,5-10 mM Mg strongly increases total synthesis of phytoalexins, affecting levels in the root tissue and the medium. The antagonistic effect of Ca and Mg ions on phytoalexin synthesis, accumulation and release by soybean roots is discussed.

PLA 182

The effect of sulfur starvation on cadmium treated maize plants

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Plants treated with cadmium produce high amounts of sulfur rich heavy metal sequestring polypeptides, called phytochelatins. In former investigations we could show, that the high need for reduced sulfur compounds stimulates the pathway of sulfur assimilation in maize roots.
In the present studies we analyzed the effect of sulfur starvation on cadmium treated plants. Maize seedlings were grown on sulfur free nutrient solution for 10 days before cadmium was added to a final concentration of 100 µM. 3 to 5 days later, the roots were analyzed with respect to their content in cadmium binding polypeptides, using HPLC techniques. Almost no such compounds could be detected. The treated plants showed an accelerated senescence compared to control plants, which could be due to the fact, that cadmium can not be chelated and detoxified in the root system of these plants and is therefore transported to the shoots where it causes the observed effect.

PLA 183

EFFECT OF CADMIUM ON SULFATE ASSIMILATION IN THE LICHEN PARMELIA SULCATA AND ITS PHYCOBIONT

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In the city of Biel(Switzerland), we measured under natural conditions up to 3 ppm Cadmium (Cd) in the lichen Parmelia sulcata. Since plants treated with Cd produce heavy metal binding peptides with high cysteine content, we thought that Cd could also stimulate assimilatory sulfate reduction in lichens. To test this idea we cultivated P. sulcata in a nutrient solution containing 13 ppm Cd and ^{35}S-sulfate as sulfur source. No increased incorporation of label into the protein and aminoacid fraction could be detected within 24 and 48 hours, as compared to controls. No effect was observed in the algal fraction of the lichen as well. When Trebouxia, the Phycobiont of P. sulcata, was cultivated under the same conditions for 48 hours a 100% increase in the amount of (^{35}S) label in the protein and aminoacid fraction was observed. These preliminary results indicate that the mycobiont protects the phycobiont from being affected by Cd.

PLA 184

SEPARATION OF STROMAL AND ENVELOPE MEMBRANE PROTEINS OF SPINACH CHLOROPLASTS INTO HYDROPHILIC AND LIPOPHILIC FRACTIONS

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The purpose of this investigation is to characterize and differentiate between the proteins of the chloroplast stroma and inner (IM) and outer (OM) envelope membranes which are encoded by the chloroplast genome. To this aim, protein synthesis was carried out in intact chloroplasts under controlled conditions in the presence of ^{35}S-methionine as protein precursor. Stroma and membrane proteins were separated according to their hydrophobic or hydrophylic (or more loosely bound) properties by Triton X-114 phase partition. Proteins of the IM fraction are mostly found in the detergent phase while the OM proteins are essentially present in the aqueous phase. Only a few stromal proteins in the range of 45 to 65 kD are recovered in the organic fraction. Among the proteins coded by c-DNA, the 75 and 45 kD stromal proteins, the 36 kD protein of the IM and the 33 and 16 kD proteins of the OM are strongly labeled. 2D-electrophoresis and immunological techniques have been used to characterize further the localization of these proteins.

PLA 185

THE PRIMARY STRUCTURE OF SPINACH CHLOROPLAST THIOREDOXIN F

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Chloroplasts contain two different types of thioredoxins (Td) involved in enzyme regulation, called Td f and m depending on their target enzymes. We already reported the complete amino acid sequence of the Td m isomers from spinach chloroplasts and have now determined the primary structure of Td f by : 1) conventional sequencing including fragmentation, Edman degradation and carboxypeptidase digestion of the protein, 2) isolation and analysis of cDNA clones coding for Td f. Only one form of Td f, MW 12564, containing 113 amino acids, was found. Its amino terminal Met is blocked probably by methylation. Apart from the active site Td f shows little homology with other Tds (24% with Td m, 21% with Td from E.coli) and differs by the presence of a third cysteine in the C-terminal part of the sequence. However analysis of the sequence shows that important residues along the polypeptide chain are conserved and suggests that the secondary structure is quite similar to the one of E.coli Td.

PLA 186

IS ATP LEVEL A PHYSIOLOGICAL AGE INDICATOR IN POTATO TUBERS, APPLES AND PEARS DURING STORAGE ?

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The yield of potato crops depends on several factors among which one of the most important is the physiological age of seed tubers. Levels of proteins, sucrose, nucleic, citric and malic acids have been proposed as indicators of physiological age. The ATP level, a potentially interesting indicator, was enzymatically (Luciferase) determined in seed potato tubers (cv. UKAMA) to monitor the changes occuring in the energy metabolism during storage. Results show that the concentration of ATP which was about 12 µg/g fresh weight in September 1986 increased to reach a first peak in October (dormancy break) then a second peak in January 1987 (maximal sprout growth). The first and second peak levels were about 2 and 5 times higher than the initial value measured in September. In March 1987, about one month before tuberisation, the ATP content dropped drastically. Similar results were obtained with other potato cultivars. In conclusion, ATP level appears to be a suitable indicator of the physiological age of potatos, as well as of apples and pears.

PLA 187

ENDOGENOUS ELEMENTAL SULFUR (S^o) DURING GERMINATION OF PHOMOPSIS VITICOLA α-SPORES

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Endogenous S^o plays a fundamental role in the regulation of dormancy and aging of P. viticola α-spores. Endogenous S^o, especially mitochondrial S^o, because of its high capacity to oxidize sulfhydryl groups exerts an inhibitory action on mitochondrial respiratory activities, and is likely responsible for the maintenance of dormancy of α-spores [Beffa et al. Physiol. Plant. 69,443-450 (1987), (1988, in press)].
We have measured by HPLC the content of S^o during germination of α-spores. In the early stages of germination the disappearance of S^o is directly related to the dramatic increase of respiratory activity. This was followed by an increase of ATP level and the synthesis of DNA, RNA, proteins and lipids. It is suggested that the reduction of endogenous S^o at the level of mitochondrial respiratory chain, with hydrogen sulfide production, is responsible for the breaking of dormancy and the stimulation of metabolism indispensible for the α-spore germination.

PLA 188

INDUCED LOCAL RESISTANCE OF WINTER WHEAT TO ERYSIPHE GRAMINIS f.sp.TRITICI: cDNA CLONING AND IN VITRO TRANSCRIPTION

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As molecular biological approach to find and characterize possible host coded resistance genes, we did a cDNA cloning of wheat mRNA from plants with induced local resistance to Erysiphe graminis f.sp.tritici.
We have found that a primary infection of young wheat seedlings with the incompatible pathogen Erysiphe graminis f. sp.hordei induced partial (60 - 70%) local resistance to challenge infection 12 h later with the compatible pathogen. mRNA was extracted from induced resistant first leaves and a cDNA library was established in lambda ZAP. Differential screening of the library (induced resistant versus non-infected plants) resulted in isolation of several cDNA clones that correspond to induced mRNA species, as shown by Northern blot analysis.
^{32}P-labelled in vitro transcripts of nuclei isolated from infected first leaves were hybridized to cDNA dot blots.

PLA 190

Kinetics of pigment biosynthesis during development of flowers in Portulaca grandiflora (Hook.)

Trezzini G.F. & Zryd J.-P., Laboratoire de phytogénétique cellulaire, UNIL, CH-1015 LAUSANNE.

In order to check for the presence of mRNAs and enzymes responsible for the biosynthesis of betalain type pigments, we analysed by HPLC the kinetics of pigments and precursors (Tyr, DOPA) accumulation during flower bud development of magenta, red and pale yellow phenotypes. The time between first analysis and flowering is about 5 days. At day 0, when petals are still uncolored, DOPA accumulates. This precursor disappears when the first pigment is formed. This first pigment is a yet not described yellow pigment, present in all analyzed phenotypes. The amino acid moiety of the molecule is tyrosin; we propose the name **portulacaxanthin II** for this new pigment. At day 2-3, betanin (the main magenta pigment in P. grandiflora) appears in the petals of magenta and red flowering phenotypes.

PLA 191

REGULATION OF BETALAINS BIOSYNTHESIS IN RED BEET CELLS IN CULTURE

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To study the regulation of secondary metabolites in plant cells in culture, we developped a model system based on the red beet alcaloids : betalains. Different cell strains were established, displaying green (uncolored), yellow, orange, red and violet pigmentation respectively. Occurence of these basic phenotypes appear to be strictly related to the composition of the media used for cultivation. Certain interconversions were also observed but they occur only between closely related phenotypes, that had been set up under similar culture conditions. We postulate them to have followed similar developmental pathways. Certain phenotypes behaved as though they were separate **clonal species**. Under inducing condition, phenotypic changes appear to be stimulated by the presence of 5-azacytidine and delayed by 3-methoxybenzamide, two factors known to affect differentiation processes. We have now isolated some of the proteins involved in the synthesis of betaxanthins, starting with an orange strain. Aminoterminal sequences are still under determination in order to prepare oligonucleotides sequences to screen a cDNA library constructed with the poly(A)+RNA.

PLA 192

EFFECTS OF PHLOEM INTERRUPTION ON LEAF SENESCENCE IN WHEAT.

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Field-grown wheat plants were treated at anthesis with steam either at the base of the flag leaf (A) or below the ear (B) in order to interrupt the phloem (steam girdling). The flag leaf senesced rapidly after interruption of the export through the phloem in treatment A: More than 50% of the proteins and chlorophylls were degraded within 7 days, while free amino groups increased to the 3-fold level and remained high until day 21. Carbohydrates accumulated during the first 7 days and decreased thereafter. Enzyme patterns changed earlier than in control plants (decreased activities of assimilatory enzymes; increased endopeptidase activities). In treatment B (steam girdling on the stem below the ear) flag leaf senescence was delayed compared to the control. Assimilatory enzymes (nitrate reductase, glutamine synthetase, phosphoribulokinase) remained active longer. The contents of carbohydrates and free amino groups were slightly enhanced between day 14 to 35. Source/sink relations and the redistribution of nutrients and metabolites through the phloem must be considered as important factors for the control of senescence in a particular leaf.

PLA 193

LACCASE PRODUCTION ASSOCIATED WITH VIRULENCE OF **ENDOTHIA PARASITICA**

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Natural recovery of European chestnut (Castanea sativa) from chestnut blight has been attributed to the occurrence of hypovirulent (H) strains of **Endothia parasitica**. These strains contain double-stranded RNA (dsRNA), presumably of viral origin, which is not present in virulent (V) strains. Searching for physiological differences between V and H strains, we grew the strains on malt extract agar containing tannic acid (Bavendamm test). All V strains produced a strong colour reaction indicating phenol oxidase activity whereas H strains showed weak or no activity. Transfer of dsRNA into V strains by hyphal anastomosis resulted in transfer of hypovirulence and reduction of phenol oxidase activity. Phenol oxidase is secreted into the medium at the advancing edge of the fungal colony. The enzyme was identified as phenol oxidase of the laccase type (p-diphenol: oxygen oxidoreductase, EC 1.10.3.2.) according to substrate specificity and spectrum of inhibitors. The results suggest a role for extracellular laccase in pathogenicity of **Endothia parasitica**.

PLA 194

REGULATION OF ASSIMILATORY SULFATE REDUCTION IN CULTURES OF PEA ROOTS BY CADMIUM

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Plants cultivated with Camdium (Cd) synthesize large amounts of heavy metal binding peptides, called phytochelatins (PC). Since PC have a high cysteine content and are localized mainly in the root system, we were prompted to examine, whether the enzymes of cysteine synthesis were increased in isolated pea roots cultivated with Cd. Indeed, the activity of adenosine 5'-phosphosulfate sulfotransferase (APSSTase), a key enzyme of cysteine synthesis, was up to 10 times higher in these roots as compared to controls. Addition of inhibitors of ethylene synthesis (1 mM $CoCl_2$ or 50 µM AVG) completely prevented this Cd induced increase. This inhibitory effect was reversed by ethrel, a compound forming ethylene. Addition of ethrel alone also induced an increase in APSSTase activity. Our results taken together indicate that ethylene is involved in the regulation of this enzyme activity in pea roots cultivated with Cd.

PLA 195

INFLUENCE OF O_3 EXPOSURE ON Ca^{2+} TRANSPORT BY PLANT MEMBRANES

Castillo, F.J. and Heath, R.L.,Department of Botany and Plant Sciences, University of California, Riverside, Ca 92521 USA

Ozone (O_3) exposure of plant material induces the modification of membrane permeability which results in the ionic imbalance of K^+, and other ions such as Ca^{2+}, could also be affected. In order to characterize the effect of O_3 on the transport of Ca^{2+} through plant membranes, Ca^{2+} uptake and efflux was measured in sealed membrane vesicles isolated from Pinto bean leaves from air filtered and O_3 exposed plants. Vesicles were prepared and loaded with $^{45}Ca^{2+}$ in the presence of Mg-ATP for 20 min. Then, EGTA at 100 times the Ca^{2+}concentration in the media, was added to the incubation medium and the remaining $^{45}Ca^{2+}$inside the vesicles was determined 20 min later. Active influx and passive efflux of Ca^{2+}through isolated membrane vesicles are affected by oxidative stress. Sealed vesicles accumulate $^{45}Ca^{2+}$ in the presence of ATP. Ca^{2+} uptake is stimulated about 25% in membrane vesicles from O_3-exposed leaves, compared to that in vesicles from non-exposed leaves. The addition of EGTA results in the release of the previously accumulated Ca^{2+}. Vesicles from exposed leaves release Ca^{2+}much more rapidly than do those from control leaves. These results suggest that the passive influx of Ca^{2+}into the cell in vivo can be altered by O_3 exposure, resulting in an increase of the free Ca^{2+}concentration in the cytoplasm.

PLA 196

PROTEIN CONTENT, GROWTH AND GRAVIREACTION IN MAIZE ROOTS

Overney, S. and Pilet, P.E., Institut de Biologie et de Physiologie végétales de l'Université, CH-Lausanne

Cycloheximide has been used to modulate protein biosynthesis in primary roots of Zea mays LG 11. It was used at concentrations varying from 10^{-3}M to 10^{-6}M. No differences were obtained between the protein patterns of these roots though there growth and gravireaction were both inhibited. It has to be noticed, as described by many investigators, that cycloheximide may affect cell metabolism other than by inhibiting protein biosynthesis.
When total amount of protein along the root axis was analysed, differences in the main zones (cap, elongation and differenciation zones) were always observed. Electrophoresis techniques showed that the differences obtained are both quantitative and qualitative.
^{3}H-Leucine was used to study which specific proteins are involved.

PLA 197

IMPLICATION OF ENDOGENOUS ABSCISIC AND INDOL-3YL-ACETIC ACIDS IN ZEATIN MEDIATED INHIBITION OF MAIZE ROOT GROWTH

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Application of zeatin (Z) at 10^{-5}M on primary roots of Zea mays L. lead to an inhibition of root growth. Two groups of roots, growing significantly slowly and rapidly were defined. Results showed that Z preferentially inhibited relative elongation of slowly growing roots. Since a negative correlation exists between root growth and levels of abscisic acid (ABA) and indol-3yl-acetic acid (IAA) in the elongation zone, endogenous content of ABA and IAA was measured after Z treatment. Using preparative high-performance-liquid-chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS), it was found that Z sharply enhanced ABA level (to a lesser extent for IAA) in the elongating zone of slowly growing roots while the hormone levels in rapidly growing roots seemed to be unaffected. The relationship between endogenous ABA and IAA root growth and Z effect will be discussed.

PLA 198

AUXIN AND CALCIUM EFFECTS ON ETHYLENE PRODUCTION AND 1-AMINOCYCLOPROPANE-1-CARBOXYLIC ACID CONTENT OF MAIZE ROOTS

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Interactions between several growth regulators, including ethylene and IAA (indol-3yl acetic acid), play a major role in the control of root growth. Ethylene production in roots is promoted by IAA and both compounds can inhibit root growth. This growth-inhibition may be regulated by calcium. In order to test this hypothesis, ethylene production and the content of ACC (1-aminocyclopropane-1-carboxylic acid), the immediate precursor of ethylene, were measured in maize roots treated with IAA (5.10^{-6}M). Both ethylene production and ACC content were greatly enhanced by IAA treatment. When Ca^{2+}(5mM) was applied to roots only a small increase in ethylene production was observed and the ACC content did not change or decreased slightly. The relationship between the growth-regulating activities of auxin, ethylene and Ca^{2+} in roots will be discussed.

PLA 199

SOLUBILISATION AND RECONSTITUTION OF THE PLASMALEMMA H^+ ATPase

Henry, H. and Pilet, P.E., Institut de Biologie et de Physiologie végétales de l'Université, CH-1015 Lausanne

When the proton pumping activity was essayed on membrane fractions prepared from roots of Zea mays only the tonoplast was able to pump proton in vitro. The plasmalemma was prepared as right-side-out vesicles which did not allow the exogenous ATP to reach the catalytic sites of the pumps. This was shown by the high latency of the vanadate sensitive ATPase activity. A method to analyze the plasmalemma H^+ ATPase was to change the orientation of the vesicles. This was possible by the solubilisation and the reconstitution of the pumps into proteoliposomes.
Plasmalemma was purified with linear sucrose gradients. The crude membranes preparation was first separated in 10 KP and 10 KS fractions. For the experimental conditions used, the 10 KP fraction was found devoid of the tonoplast H^+ ATPase. The plasmalemma fractions were treated with the detergent deoxycholate and the pumps were incorporated into the proteoliposomes. The ability of the reconstituted plasmalemma to pump protons was tested in vitro and analyzed into different parts of the root.

PLA 200

ABA AND IAA CONTENT AND ABA METABOLISM IN ZEA MAYS ROOT PROTOPLASTS

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In protoplasts isolated from several root tip zones of Zea Mays L. (cv. LG11), including the cap and the apex, level of indol-3-yl-acetic (IAA) and abscisic acid (ABA) and their hydrolysable conjugates was determined using for separation high performance liquid chromatography (HPLC) and for quantification, gas chromatography-mass spectrometry (GC-MS) methods. Content of free compounds was found to be maximal in protoplasts from the cap and the apex. Hydrolysable conjugated compounds were preferentially accumulated in basal root parts. Results for isolated cells from the same root zones were compared to those related to protoplasts. When DL-cis,trans,^{3}H-ABA was applied to protoplasts, ABA glucose ester, phaseic acid, dihydrophaseic acid and DPA glucoside have been detected. The intensity of metabolisation appears to be very less effective in protoplasts than in entire root segments.

PLA 201

PROPERTIES OF A CARRIER FOR IAA EFFLUX IN MAIZE ROOTS

Martin, H.V. and Pilet, P.E., Institut de Biologie et de Physiologie végétales de l'Université, 1015 Lausanne

A carrier for the efflux of IAA (indol-3yl-acetic acid) across the plasmalemma is studied. This carrier is thought to be responsible for the polarity of IAA transport, and its physiological importance appears to extend to a role in the control of tropisms, the movement of calcium, and the polarity and permeability of membranes. NPA (N-1-naphthylphthalamic acid) can be used to block its activity. The carrier was found to be preferentially localised in the apical part of the root. Although the kinetic properties of (^{3}H)NPA binding suggest that the carrier is present at 4 pmol/g F.W., calculations based on studies of (^{3}H)IAA efflux show that the carrier will be capable of changing IAA levels in the elongation zone by less than 5 % per min. The addition of auxins to the efflux medium had considerable effects on the efflux of (^{3}H)IAA, suggesting that the control of the carrier is complex. The physiological role of the IAA efflux carrier will be discussed.

PLA 202

THE IMPORTANCE OF IAA METABOLISM IN MAIZE ROOTS TREATED WITH IAA

Meuwly, Ph. and Pilet, P.E., Institut de Biologie et de Physiologie de l'Université. CH-1015 Lausanne

In order to characterize the importance of indole-3-acetic acid (IAA) metabolism during growth, intact primary Zea mays roots and apical segments were immersed in IAA at various concentrations inducing growth inhibition. IAA-loaded resin beads were also used for local treatment along intact roots. Major putative IAA metabolites and conjugates were separated on a single HPLC gradient and analysis of crude extracts was performed. The amount of IAA taken up was in all cases time-dependent during 6h and the highest values were obtained with the immersion technique. The pattern of IAA metabolites differed for intact roots and for segments, the traumatism due to cutting inducing some changes of the "normal" metabolic pathways. Furthermore, the higher the uptake of IAA the faster the metabolism. The beads allow precise application of IAA at better physiological amounts. The rate of IAA metabolism seemed to depend upon the bead location along the elongation zone.

PLA 203

QUANTITATIVE METHODS FOR THE ESTIMATION OF NUCLEAR DNA LEVELS IN ROOT CELLS : A CRITICAL ANALYSIS

Müller, M.L. and Pilet, P.E., Institut de Biologie et de Physiologie végétales de l'Université, CH-1015 Lausanne

Growth is closely related to the cell cycle in meristematic zones of plant organs.
The DNA content of a nucleus gives an indication of its position within the cycle. The DNA amplification phenomenon is studied in maize roots. Nuclei of meristems, elongation zone, stele and cortex of the differentiation zone are analysed by several methods.
The techniques in use for the determination of the DNA content are microphotometry, microfluorometry and flow cytometry.
Analysis material is obtained by preparing nuclear fractions, protoplasts or by using histological preparations. Isolated nuclei and protoplasts are stained with fluorochromes like DAPI (4',6'-diamidino-2-phenylindole), ethidium bromide and propidium iodide. Histological preparations are stained either by the Feulgen reaction for absorbance measurements or with fluorochromes. The nuclear DNA content of different zones of the root will be discussed in relation to the differentiation of the tissues.

Poster Session, Friday

Anatomy, Histology, Embryology (AHE)

AHE 204

QUANTITATIVE ANALYSIS OF FETAL RAT LUNG DEVELOPMENT

Moschopulos, M., Wandel, G. and Burri, P.H., Institute of Anatomy, University of Berne, Berne, Switzerland

We studied the development of the rat lung from day 17 of gestation to 20 hours after birth. Our aim was to analyze the fate of the early tissue components and to determine the timing of the appearance of the prospective gas exchange compartment (= parenchyma). By means of quantitative light and electron microscopy we estimated volume and surface parameters of the lung compartments and their respective changes during the pseudoglandular (days 17-20), canalicular (days 21-22), and saccular stages (day 22 to after birth). The results showed that ~ 50% of the prospective parenchymal cell mass was formed on days 19 and 20 as a mantle of tubular sprouts in a densely packed mesenchyme. Because this period presented also other morphological features of early parenchymal development, we would like to emphasize that the birth of the parenchyma can already be detected in the late but still typical pseudoglandular lung. Our observations also indicate that parenchymal formation is closely related to the presence of two types of mesenchyme: a central condensed mesenchyme representing the zone of differentiation, and a peripheral loose mesenchyme representing the zone of growth.

AHE 205

TISSUE POSTS IN PULMONARY MICROVASCULATURE: A NOVEL WAY OF CAPILLARY GROWTH IN RAT AND HUMAN LUNG

Tarek, M., Haenni, B. and Burri P.H., Institute of Anatomy, University of Berne, Berne, Switzerland

In Mercox casts of the pulmonary microvasculature of growing rats, we had previously observed the presence of small holes (diameter <1µm) in sheet-like areas of the capillary bed. We hypothetized that these cylindrical cutouts could represent the first stages of new capillary meshes, and hence correspond to a novel way of capillary development avoiding the need for capillary sprouting (interstitial or intussusceptional growth, Caduff et al., 1986). In this study, we confirmed by ultrathin serial sectioning, electron microscopic analysis and 3-D computer reconstruction that the observed holes corresponded to tissue posts of various ultrastructural configurations. With increasing diameter, the post ultrastructure changed from simple endothelial bridges to fully developed capillary meshes showing myofibroblasts and pericytic cell processes in their axis. Concomitant investigations of the human growing lung yielded similar ultrastructural findings. This study supports the concept of interstitial capillary growth and suggests that it may be a common feature of mammalian lungs.

AHE 206

MORPHOMETRY OF THE BRONCHIAL TREE OF THE HAMSTER LUNG

Im Hof V., Geiser M. and Gehr P., Pneumologische Abteilung Inselspital und Anatomisches Institut der Universität, Bern.

Silicon-rubber casts of hamster lungs have been trimmed to the first gas exchanging units. They were found to consist of 15±4 dichotomously branching air conducting generations. The transition to the gas exchange units was abrupt; 1/3 of the terminal bronchioles were followed directly by alveolar ducts, 2/3 were followed by 1 generation of respiratory bronchioles. Length, diameter and branching angle were measured in 1300 air conducting branches. Length and diameter decreased non-linearly with increasing generation number. The rate of decrease was large until the 10th generation, diminishing rapidly thereafter. The dichotomous branching pattern was found to be irregular, the 2 daughter branches differing in their dimensions and branching angles. The angle between two daughter branches remained constant ($70\text{-}80^0$). The degree of irregularity decreased with increasing generation number. 80% of the larger diameters went with the smaller angles. This pattern supports a homogeneous air distribution in a lung with conducting pathways of different lengths. It deviates from the branching pattern in human lungs.

AHE 207

EFFECT OF MICROGRAVITY ON LYMPHOCYTE ACTIVATION

O. Müller, E. Hunzinger, B. Bechler and A. Cogoli
Anatom. Inst. Uni. Bern, Lab. f. Biochem., ETH Zürich

Cultures of purified human lymphocytes were activated by the mitogen concanavalin A and subjected to different gravity conditions. Two probes were flown in the ESA Biorack during the Spacelab D-1 mission, one of them under microgravity conditions (0xg), the other in a reference centrifuge installed on board and generating 1xg. Two control probes were left on ground, one of them in a centrifuge rotating at 1,4xg. Subsamples were fixed with glutaraldehyde on mission-day 1,2,3 and 4 respectively for electron microscopic examination. Except for the probe at 0xg, all others showed signs of activation, by formation of aggregates consisting of lymphocytes, lymphoblasts and macrophages. The mean lympocyte/lymphoblast ratio was 50:1 at 0xg, 4:1 in the space centrifuge, 2,5:1 on the ground and 2:1 in the ground centrifuge. The mean mitotic index of lympoblasts was 1,2% in the space centrifuge, 1,7% in the ground probe at 1xg and 2% in the ground centrifuge. No mitotic figures were detected at 0xg. The level of cell degeneration was highest at 0xg and lowest at 1,4xg, but even at mission-day 4 at 0xg there were many lymphocytes showing normal ultrastructure.

AHE 208

TRANSLATIONAL ACTIVATION OF A DORMANT mRNA DURING MEIOTIC MATURATION OF MOUSE OOCYTES

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Institute of Histology and Embryology and Department of Pathology University of Geneva Medical School 1211 Geneva 4.

During their growth phase, mouse oocytes accumulate a mRNA species coding for tissue-type plasminogen activator (t-PA). We have detected t-PA mRNA in single fully grown primary oocytes; each oocyte contains approximately 10.000 t-PA mRNA molecules, that are localized in the cytoplasm. In growing oocytes as well as in fully grown oocytes this mRNA appears to be in a dormant, untranslated state. Resumption of meiosis triggers the synthesis of t-PA. Concomitantly, t-PA mRNA undergoes a concerted and progressive increase in size, that results, at least in part, from increased polyadenylation at the 3' end of the molecule. At the end of maturation, i.e. after ovulation, t-PA mRNA is degraded so that it is no longer detectable in old secondary oocytes or in fertilized eggs. Taken together these results show that t-PA mRNA is the first mRNA under translational control to be identified in mammalian oocytes. By injecting truncated or chimaeric synthetic mRNAs into primary oocytes we are at present attempting to identify the sequences that define t-PA mRNA as maternal.

References: Cell, 43:551-558; Genes & Development, (in press).

AHE 209

MUSCULAR PLASTICITY AND REINNERVATION CAPACITY AFTER REINNERVATION BY A FOREIGN NERVE

Käser, L., Institute of Anatomy, University of Zürich-Irchel, CH-8057 Zürich (Sponsor: M. Müntener)

In male rats the denervated sternomastoid muscle was either foreign-reinnervated by the 5fold smaller omohyoid nerve or self-reinnervated by its original nerve. After survival times of 7, 8, 9 or 10 months, nerves and muscles were investigated histochemically (ATPase, cytochrome C-oxidase, AChE) and immunohistochemically (parvalbumin). The omohyoid nerve could completely reinnervate the 3fold amount of muscle fibers. After 7 months the self-reinnervated sternomastoid muscle revealed a very low portion of IIB-fibers (23%), which remained so during the period investigated, whereas in the foreign-reinnervated muscle the number of this fiber type strongly increased at the expense of type IIA-fibers until month 9 (from 24 to 63%). This fiber pattern remained constant afterwards. The experiments demonstrate that after foreign-reinnervation of additional muscle tissue remodeling processes at the neuromuscular synapse are ongoing for a long time.

AHE 210

PROTEOGLYCANS OF NORMAL AND NEOPLASTIC HUMAN MAMMARY TISSUES
Alini,M., Losa,A.G., Laboratorio di Patologia Cellulare, CH-6600 Locarno

The biochemical composition and the ultrastructural distribution of proteoglycans(PG)was investigated in the extracellular matrix(ECM)of human breast tissues with benign lesions and with invasive carcinoma or metastasis.PG were extracted from breast tissues under dissociative conditions(4 M Guanidinium Chloride),isolated by CsCl gradient ultracentrifugation,and purified by exclusion and affinity chromatography. Biochemical data showed that the rate of high density and high molecular size PG was higher in neoplastic than in normal tissues.Further,enzymatic analysis(chondroitinase ABC and AC)and notrous acid degradation,revealed a dramatic change in the ratio of glycosoaminoglycans side chains of PG between neoplastic and normal tissues.Particularly,an increased content of chondroitin sulfate(56% vs 34%)and heparan sulfate(28% vs 18%)was found in neoplastic versus normal tissues,whereas dermatan sulfate content's decreased(16% vs 48%).These data are in line with morphometric results performed on intact tissues,showing that tumoral PG are 2 to 3 times longer than normal PG.Thus,the ultrastructural and biochemical changes of PG point to altered properties in the ECM of neoplastic breast tissues.

AHE 211

REVERSAL OF BILE-SALT SECRETORY POLARITY IN RAT HEPATOCYTES FOLLOWING BILE-DUCT LIGATION

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Obstructive cholestasis is associated with various hepatocellular membrane alterations (Sem.Liv.Dis. 6,233,1986). We therefore studied the effect of bile duct ligation on the canalicular 100kDa bile salt carrier (cBSC) (J.Biol.Chem. 262, 11324,1987). Monospecific anti-cBSC antibodies were used to identify and quantitate cBSC in intact liver, isolated plasma membrane vesicles and bile. Furthermore, anion transport was compared in basolateral (blLPM) and canalicular (cLPM) vesicles. 50 hours of cholestasis resulted in a release of cBSC into bile. Immunofluorescence showed an overall decrease of cBSC in the canalicular membrane associated with a novel accumulation in the basolateral membrane. This redistribution of cBSC was functionally paralleled by an increased Na^+ independent electrogenic taurocholate anion uptake in blLPM vesicles while "cholestatic" cLPM vesicles exhibited lower electrogenic taurocholate anion transport than "normal" ones.These results demonstrate that a significant portion of functionally active cBSC is redistributed and/or newly inserted into the basolateral plasma membrane.

AHE 212

THE REACTIVITY OF HUMAN TUMOUR SAMPLES TO ANTI-CYTOKERATIN AND TO ANTI-MACROPHAGE Mabs RELATED TO THEIR HISTOPATHOLOGICAL DIFFERENTIATION
Sossai R., Cappelli-Gotzos B., Gotzos V., Isoz V. and Conti G., Institut d'Histologie, Université Pérolles, CH-1700 Fribourg

It is known that human carcinomas haven't a constant pattern in cytokeratin expression. Is this variability differentiation-related ? Is the macrophage infiltration related to the histopathological differentiation ? Cryostat sections of fresh human pulmonary and digestive tumour samples were incubated with anti-human-cytokeratin and anti-human-macrophage Mabs (DAKO). Histopathological grading was established on paraffin sections stained with four usual histological stainings.
According to our preliminary results it seems that variability in cytokeratin expression is rather due to cell heterogeneity inside one tumour, than to the histopathological differentiation of the tumour. The results of the evaluation of macrophage infiltration will be discussed in details on the poster.

Pharmacology, Toxicology (PHA)

PHA 213

TRANSPORT OF ORGANIC ANIONS BY BRUSH BORDER MEMBRANE VESICLES (BBMV) OF THE PIG KIDNEY.
D. Werner, F. Martinez, C. Schäli, C. Montrose-Rafizadeh and F. Roch-Ramel. Institut de Pharmacologie de l'Université, CH-1005 Lausanne.

Rats and dogs (species reabsorbing urate) have a renal proximal luminal anion exchanger with affinity for urate, p-aminohippurate (PAH), lactate, OH^- and Cl^-. We investigated if such a mechanism also exists in BBMV of a species secreting urate like the pig.
In pig BBMV, ^{14}C-urate and ^{3}H-PAH uptakes were not stimulated by an outwardly-directed OH^- gradient. However ^{3}H-PAH uptake was stimulated by an outwardly-directed Cl^- gradient, but a voltage clamp abolished this stimulation, suggesting an influence of electrical potential on ^{3}H-PAH transport. Creation of an electropositive intravesicular space by an inwardly-directed K^+ gradient in presence of valinomycin stimulated ^{3}H-PAH and ^{14}C-urate accumulation, with their 4 s uptakes being inhibited by probenecid (10^{-3} M). In addition, 4 s ^{3}H-PAH uptake was saturable.
These results suggest that, in the pig, the luminal step of PAH and urate secretion does not use an anion exchanger but rather a facilitated mechanism driven by an electrical potential.

PHA 214

EFFECTS OF ALDOSTERONE AND 19-nor-ALDOSTERONE ANALOGS ON TRANSEPITHELIAL SODIUM TRANSPORT

Gaeggeler H.P., Harnik, M., and Rossier, B.C., Institut de Pharmacologie de l'Université, CH-1005-Lausanne,Switzerland and Dept of Biotechnology, Tel Aviv University, Israel

19-nor-Aldosterone was recently shown to be a potent mineralocorticoid agonist in the mammalian kidney and the amphibian urinary bladder. In the present study, we have investigated the potential agonist/antagonist activity of 19-nor-aldosterone, 19-OH-aldosterone and 18-deoxy-19-nor-aldosterone on transepithelial sodium transport measured by the short-circuit current method in the urinary bladder of the toad (Bufo marinus) in vitro. The order of potency was the following: Aldosterone>19-OH-aldosterone>19-nor-aldosterone while 18-deoxy-19-nor-aldosterone had no agonist activity up to 3µM. By contrast, 18-deoxy-19-nor-aldosterone inhibited aldosterone-dependent sodium transport (10nM) in a dose-dependent manner. At an antagonist/agonist molar ratio of 100 to 1, sodium transport was inhibited about 50%, making this compound more potent than the reference antagonist drug spironolactone.

PHA 215

OUABAIN-SENSITIVE SODIUM TRANSPORT AND OUABAIN BINDING SITES IN A6 KIDNEY CELLS

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Aldosterone increases transepithelial sodium transport and the relative rate of synthesis of Na,K-ATPase in the A6 cultured cell derived from X.laevis kidney. In order to assess the activity of the sodium pump at the cell surface, we measured: 1) ouabain-sensitive sodium transport in A6 cells grown on collagen coated filters and 2) binding of ^{3}H-ouabain to intact cells grown on plastic petri dishes. In the sodium transport assay, we observed a Ki for ouabain ranging from 70nM to 300nM which was not significantly affected by aldosterone. In the binding assay the Kd for ouabain was about 30nM and the estimated Nmax was 10^5 sites per cell.
We are presently studying the effects of aldosterone on Kd and Nmax in cells grown on plastic petri dishes and in cells grown on collagen-coated filters.

PHA 216

TRANSPORT OF TETRAETHYLAMMONIUM (TEA) BY RABBIT RENAL BASO-LATERAL MEMBRANE (BLM) VESICLES. C. Montrose-Rafizadeh, B. Guisan, H. Murer, F. Roch-Ramel. Institut de Pharmacologie de l'Université, 1005 Lausanne.

The mechanisms involved in the transport of the organic cation, TEA, across the BLM of renal proximal tubule of rabbit were investigated using isolated membrane vesicles. With membrane potential held near zero, preloading the vesicles with 10 mM of unlabeled TEA stimulated the 15 s uptake of 14C-TEA when compared to control vesicles by 346% and the transport demonstrated an overshoot of 3 times the equilibrium value. The 1 min uptake of 14C-TEA (0.4 mM) was inhibited 70%, 65%, 55% and 28% by 1 mM, 0.2 mM, 0.1 mM and 0.01 mM mepiperphenidol respectively. Beside this exchange mechanism for TEA transport, we observed that changes in the membrane potential could influence the TEA transport. The TEA uptake was increased when the interior of the vesicles was negatively charged compared to the medium by an outwardly directed K^+ gradient and valinomycin. Moreover this transport showed an overshoot of 2 times the equilibrium value. These data demonstrate the presence in the rabbit renal BLM of an exchange mechanism for TEA in a voltage clamped preparation, and also show that changes in the membrane potential affect net TEA transport.

PHA 217

EFFECT OF TUNICAMYCIN ON INTRACELLULAR TRANSPORT AND FUNCTIONAL PROPERTIES OF Na,K-ATPase

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Na,K-ATPase is made of a catalytic α-subunit (Sα) and a glycosylated β-subunit (Sβ) of unknown function. We have studied the effect of the coreglycosylation inhibitor tunicamycin (TM) on Na,K-ATPase in toad urinary bladder (TBM) cells grown on collagen-coated filters. Pretreatment of cells for 21 h with 1µg/ml TM resulted 1) in a complete inhibition of Sβ glycosylation and 2) in a 30% decrease of the cellular pool of Sα reflecting a 70% decrease in the accumulation of newly synthesized Sα and Sβ. As assessed by surface labeling of intact cells, the non-glycosylated Sβ was expressed at the basolateral cell membrane. Ouabain-inhibitable Na,K-ATPase activity measured in cell homogenates of TM treated cells (up to 42 h) decreased more rapidly than the cellular Sα pool, but in parallel with the decrease in Na^+ transport at the basolateral membrane measured in amphotericin B (12 µg/ml) treated cells. The results suggest that inhibition of Sβ glycosylation does not impede intracellular transport of the Na,K-ATPase but abolishes its enzyme and sodium transport activity.

PHA 218

PASSIVE PERMEABILITY OF ^{14}C-SALICYLATE (Sal) IN THE RABBIT CORTICAL COLLECTING TUBULE (CCT)

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At urine pH below 7.5, salicylate undergoes net reabsorption in the nephron presumably by passive backdiffusion. We investigated the characteristic of nonionic backdiffusion of Sal (J_{Sal} in fmol/mm.min) at 23°C in isolated perfused rabbit CCT, using fixed buffers for perfusion (MES 20 mM) and bath (HEPES 20mM). Bath was bubbled with O_2 and perfusate contained about 0.1 mM Sal. When bath pH was maintained at 7.4, J_{Sal} was inversely related to luminal pH. At luminal pH of 7.4, J_{Sal} was -2.3±6.1 (n=4), and increased to 25.1±10.2 (n=5), 52.1±20.2 (n=5), and 123.4±23.2 at luminal pH respectively of 6.0, 5.5 and 5.0. Total(nonionized and ionized) Sal apparent permeability (10^{-6} cm/s) increased in parallel : 6.2±1.1 (n=10), 17.2±0.5 (n=5), 38.8±0.4 (n=9). However this permeability was constant and averaged 4352±458 (n=13) when only the nonionized moiety of Sal was considered. J_{Sal} showed flow-rate dependency but no relationships with spontaneous transepithelial voltage. In confirmation of the theory of nonionic diffusion, these results indicate that only the nonionized moiety of Sal can cross the CCT epithelium.

PHA 219

STIMULATION BY BOVINE SERUM ALBUMIN (BSA) OF RENAL SECRETION OF ORGANIC ANIONS.

K. Besseghir, I. Gautschi and F. Roch-Ramel, Institut de Pharmacologie, Bugnon 27, 1005 Lausanne.

To determine the role of BSA binding in the net renal secretion of p-aminohippurate (J_{PAH} in fmoles/mm.min, 20% bound to 1% BSA) J_{PAH} was measured in *in vitro* isolated perfused rabbit proximal S_2 segments. At bath PAH of about 1 µM, and in the absence of BSA, J_{PAH} was low (3.8±1.2, n=4) and was reversibly 8-fold stimulated by the addition of 1% dialyzed defatted BSA, to 30.5±3.1 ($p<0.001$)). 1% dextran 40 did not change J_{PAH}. Albumin effect on J_{PAH} was dose-dependent and reached a plateau at 1% BSA. Preliminary data indicate that 1% BSA decreased the apparent Km (39 vs 250 µM) with minor modifications of the apparent Vmax of J_{PAH}. A comparable stimulation of transport was seen with another organic anion, methotrexate, while no effect of BSA was observed with the organic cation N^1-methylnicotinamide. These results indicate that albumin could promote selectively organic anion transport by the proximal cell.

PHA 220

METABOLIC FUNCTION OF PRIMARY RAT HEPATOCYTES IN LONG-TERM CULTURES: COMPARISON BETWEEN PURE CULTURE AND CO-CULTURE WITH RAT LIVER EPITHELIAL CELLS.

Baitella-Eberle, G., Gantner, D., Maier P., Institut für Toxikologie, ETHZ und Universität Zürich, CH-8603 Schwerzenbach.

The morphology, viability and liver specific functions were determined in primary pure rat-hepatocyte cultures and compared to hepatocytes in co-cultures with rat liver epithelial cells (RLEC) isolated and cultured from 10 day old rats. In co-cultures, the hepatocytes' specific morphology was maintained for more than 20 days, cell detachment (acute toxicity) was strongly reduced (< 5% within 10 days), and no loss of viability was observed over 20 days. The cellular protein content, the activity of lactate-dehydrogenase as well as the cytochrome P-450 dependent epoxidation of aldrine to dieldrine (aldrine epoxidase) were enhanced nearly two fold compared to pure cultures. Our experiments with two morphologically different RLEC-lines suggest that co-cultures may be a tool to maintain an in vivo-like metabolic behaviour of hepatocytes in vitro. Such metabolic capabilities are a prerequisite in the development of in vitro toxicity tests.

PHA 221

CYTOCHROME P-450 ISOZYMES IN RAT OESOPHAGUS WITH HIGH SUBSTRATE SPECIFICITY FOR ASYMMETRIC NITROSAMINES

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N-Nitrosomethylamylamine and related asymmetric nitrosamines selectively induce esophageal carcinomas in laboratory rodents. There is evidence that this effect is due to organ-specific bioactivation involving a specific P-450 dependent monooxygenase (von Hofe et al., Carcinogenesis 8:1337-1341, 1987). In search for the respective P-450 isozyme, we synthesized a synthetic hydrophilic peptide, Pro-Lys-Ser-Arg-Gly-Asn-Phe-Pro-Pro-Gly-Pro-Arg-Pro-Leu-Pro-Leu, corresponding to a region near the amino terminus known to be conserved in all rodent P-450 species. Rabbit antisera raised against this peptide were used in Western blots. Strongly immunoreactive P-450 bands were detected in rat liver, oesphagus, kidney, lung and nasal mucosa. IgG fractions were coupled to Sepharose and used to partially purify esophageal P-450 by immunopurification. Release of P-450 was achieved under mild conditions using a large excess of the peptide. We currently raise antibodies to an oligopeptide corresponding to a preserved P-450 region near the C-terminus, to be used in a second immunopurification step.

PHA 222

HUMAN ANTI-LIVER/KIDNEY MICROSOME AUTOANTIBODIES (ANTI-LKM) IN CHRONIC ACTIVE HEPATITIS: DIFFERENT ANTIGENS ARE RECOGNIZED IN LIVER VERSUS KIDNEY

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In a subgroup of children with chronic active hepatitis (CAH) autoantibodies which bind to endoplasmatic reticulum of liver and kidney cells (anti-LKM1) are observed. The liver antigen was identified as P450 buf I / db1 (MW of 50 kd), the P450 involved in the genetic polymorphism of deficient oxidation of debrisoquine and numerous other drugs. Immunopurification of the microsomal anti-LKM1-antigen of kidney revealed a single band (MW 54 $\pm$ 1 kd) on SDS-PAGE. Monoclonal antibodies against P450 db1 do not recognize this protein on Western blots. These results suggest that liver and kidney anti-LKM1-antigens may be different proteins. The identity of the kidney antigen and the possible relation of both antigens to chronic active hepatitis are presently studied.

PHA 223

DEACETYLATION OF 2-ACETYLAMINOFLUORENE BY HUMAN LIVER MICROSOMES

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Competition between enzymatic pathways in the biotransformation of drugs and xenobiotics often has implications for efficacy or toxicity. 2-acetylaminofluorene (AAF) is formed from 2-aminofluorene (AF) by liver cytosolic N-acetyltransferase (NAT), and may be reconverted to AF by a microsomal deacetylase of the C-esterase class. The latter reaction is an important determinant of the carcinogenic potential of AAF in animal systems. We studied the deacetylation of AAF and a number of other arylacetamide substrates in microsomes from 24 different human livers. The distribution of the AAF-deacetylase activity in these samples revealed two livers with markedly elevated formation of AF. A 25-fold variation in the ratio of deacetylase to NAT activities was also observed, although the two activities were not correlated. Sulfamethazine was a good substrate for cytosolic NAT but its acetyl derivative was not metabolized by the deacetylase, while AF and AAF were efficiently biotransformed by the former and latter enzymes, respectively. This suggests that for different substrates, contributions from variable acetylating and deacetylating pathways may lead to variation in drug and chemical disposition in vivo.

PHA 224

INCREASED GENOTOXIC EFFECTS OF PROMUTAGENS IN DROSOPHILA MELANOGASTER STRAINS WITH ALTERED P-450 METABOLISM

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In the Somatic Mutation and Recombination Test (SMART) of Drosophila melanogaster larvae trans-heterozygous for two recessive wing markers on chromosome 3 are exposed to test compounds. Genotoxic effects resulting in homo- or hemizygous clones are indicated by spots on the adult wing (Würgler, F.E. and E. Vogel, in F.J. de Serres (ed.) Chemical Mutagens, Vol.10, Plenum Press, New York (1986) p.1-72). In order to improve the test performance with respect to promutagens the first and second chromosomes of the test strains were substituted by those of a wild type strain with constitutively increased cytochrome P-450 activities linked to a locus on the second chromosome. First experiments with four selected promutagens (aflatoxin B1, benzo(a)pyrene, diethylnitrosamine, urethane) reveal strong evidence for a clearly more efficient activation resulting in increased toxic and genotoxic effects in the new strains. Work supported by Swiss Cancer League.

PHA 225

PHARMACOKINETIC BEHAVIOR OF OBIDOXIME IN SARIN INTOXICATION ILLUSTRATED BY A TWO-COMPARTMENT OPEN MODEL IN RATS

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A pharmacokinetic study was carried out after a single iv administration of 50mg/kg of obidoxime (Toxogonin®), a drug actually used as antidote in poisoning with organophosphorus compounds, e.g. sarin. The purpose of this study was to gain more insight into the kinetic behavior of obidoxime as a single iv injection (exp.1) and as a therapeutic measure upon intoxication with sarin (exp.2) in anesthetized and artificially ventilated rats. Plasma concentration, biliary and urinary excretion as well as inulin clearance to follow kidney function, were determined during the experimental procedure. Plasma data were fitted to a two-compartment open model.-Considering the resulting pharmacokinetic parameters, marked differences were observed between the two experiments. Exp.2 showed higher plasma levels at all times and a remarkable reduction of the glomerular filtration rate.-It is concluded that the delay of elimination provoked by sarin is mainly due to hemodynamic changes.

PHA 226

OXIDANT STRESS GENERATED BY ACTIVATED KUPFFER CELLS IN PERFUSED RAT LIVER.

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A growing body of evidence indicates that Kupffer cells play an important role in some models of liver injury. The mechanisms responsible for the potentiation of injury by Kupffer cells may be related to the generation of reactive oxygen species. To test this hypothesis we measured the biliary excretion of glutathione disulfide (GSSG) in the perfused rat liver following infusion of latex beads (0.8 µ, 600 mio/liver). Biliary GSSG is a sensitive index of oxidant stress in the liver, and phagocytosis of latex beads activates Kupffer cells. Following the infusion of latex biliary GSSG increased from 6.0$\pm$1.9 ($\pm$SD, n=6) in control livers to 11.1$\pm$2.4 nmol/g.30 min. Infusion of phorbol myristate acetate (PMA), another activating agent, increased portal pressure by 5.7$\pm$2.8 and <20 mmHg at 2o and 200 nM, respectively. Bile flow stopped at 200 nM and biliary GSSG decreased, indicating major effects of PMA on hepatocyte rather than Kupffer cell function. Our data suggest that activation of Kupffer cells in the intact organ results in the formation of reactive oxygen species. The resulting oxidant stress may contribute to hepatocellular injury.

PHA 227

CHLORAMINES RATHER THAN HYDROGEN PEROXIDE ACCOUNT FOR THE OXIDANT STRESS PRODUCED BY ACTIVATED POLYMORPHONUCLEAR LEUKOCYTES (PMN) IN PERFUSED RAT LIVER.

M. Bilzer, B.H. Lauterburg, Dpt of Clinical Pharmacology, University of Berne, Berne, Switzerland.

O2- and H2O2 produced by PMN may contribute to hepatic damage in inflammatory liver disease. We, therefore developed a model to study effects of activated PMN on the intact liver. Human PMN were infused into the portal vein of the recirculating perfused rat liver after activation by exposure to phorbol myristate acetate (PMA) for 30s. Virtually all cells were trapped within the liver (10e7 PMN/g). Following infusion of activated PMN (but not resting cells or PMA alone) biliary release of oxidized glutathione (GSSG) rose to 0.52 $\pm$ 0.17 nmol/min.g liver) and returned to baseline (0.14 $\pm$ 0.09) within 60 min. This time course paralleled the O2- production by PMN in vitro and may reflect the "oxidative burst" of PMN trapped by the liver. Infusion of the amount of H2O2 (30 nmol/min.g liver) produced by 10e7 PMN did not increase biliary GSSG. In contrast, 15 µM monochloramine (NH2Cl) increased biliary GSSG efflux severalfold, whereas 15 µM H2O2 and 65 µM taurinechloramine had only slight effects. These results suggest that NH2Cl may be more important than H2O2 in the oxidant stress and cell damage produced by PMN.

PHA 228

INCREASED SYNTHESIS OF GLUTATHIONE (GSH) IN ACTIVATED POLYMORPHONUCLEAR LEUKOCYTES (PMN).

M. Bilzer, B.H.Lauterburg, Institut für Klin. Pharmakologie, Universität Bern, CH-3010 Bern

Activation of PMN results in the production of O_2^-, H_2O_2, and HOCl. GSH may play an important role in protecting the PMN and their surrounding environment against oxidative damage. In order to better understand the function of GSH in PMN we characterized GSH turnover in resting and activated PMN. Human PMN were preincubated in the presence or absence (control) of 200 µM buthionine sulfoximine (BSO), an inhibitor of GSH synthesis. After stimulation with phorbol myristate acetate (PMA) intracellular GSH (GSH + GSSG) remained stable for 20 min (12.9±2.4 nmol/10^7 PMN, n=7) and then decreased to 6.5±2.1 at 80 min. In contrast, BSO treated cells showed a rapid decrease from 12.4±2.9 to 7.9±2.2 and 1.1±0,8 nmol/10^7 PMN at 20 and 80 min, respectively. In resting cells GSH was constant for 80 min with and without BSO. Extracellular GSH and GSSG added to PMN was rapidly consumed following activation (14 nmol/10^6 PMN after 20 min). Incubation of GSH and GSSG with HOCl resulted also in a concentration dependend disappearance of both compounds. Our data indicate that GSH synthesis is stimulated in activated PMN to compensate for the loss of GSH occuring by detoxification of microbicidal oxidants.

PHA 229

RADIOMETRIC DETERMINATION OF MEPHENYTOIN HYDROXYLASE IN VITRO

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Aromatic hydroxylation of mephenytoin (M) exhibits an extensive NIH-shift of the metabolically labile tritium isotope. Thus, for radiometric in vitro investigations in respect to the underlying genetic polymorphism in man, the chemical reaction conditions for quantitative 3-H-water release from tritium carrying phenolic M metabolites after NIH-shift were studied. Racemic 3-H-mephenytoin (4-phenyl) was synthesized from the 4-Br-compound by catalytic tritiation. The purified drug (0.2 µCi/tube) was incubated with rat liver microsomes (1.5 mg prot, 60 min) followed by an isotope scrambling procedure (100°C, 30 min, 5 N HCl) for 3-H-water release. Preliminary data indicate a Km of approx. 100 µM and a Vmax of 150 pmol/mg prot x min in liver microsomes of female Sprague-Dawley rats. Thus, the quantitative release of metabolically labile 3-H-isotopes by acid treatment at elevated temperatures renders 3-H-water measurements suitable for in vitro radiometric M-hydroxylase activity determinations in hepatic and extrahepatic tissues.

PHA 230

RADIOMETRIC DETERMINATION OF OXIDATIVE DRUG POLYMORPHISM BY DEXTROMETHORPHAN-O-DEMETHYLATION IN HUMAN AUTOPSY LIVERS

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Dextromethorphan (DEM) is a well established test drug for the determination of debrisoquine-type drug hydroxylation polymorphism. The aim was therefore to study DEM-O-demethylation in microsomes of human autopsy livers and to compare it to the unimodally distributed 7-ethoxycoumarin deethylase activity as a microsomal viability test. Calcium co-sedimented microsomes were prepared from 3 g liver-pieces and radiometric determinations of 3-H-formaldehyde arising from 3-H-DEM-O-demethylation were carried out. The ratio of 7-OH-coumarin to 3-H-formaldehyde formation could represent a measure of genetic drug hydroxylation deficiency. 10 livers had ratios between 0.10-6.06 (1.7±1.7; x±SD) whereas 2 livers showed elevated ratios of 16 and of >100 and might therefore represent post mortem livers of poor metabolizer subjects. Thus, multiple cytochrome P-450 activities can be determined in autopsy material and the above described method might be a valuable tool in the assessment of the debrisoquine-type activity in human tissues.

PHA 231

DEBRISOQUINE AND MEPHENYTOIN HYDROXYLATION IN PRIMATE MONKEYS

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Debrisoquine (D) and mephenytoin (M) hydroxylation exhibit a genetic polymorphism in man. Searching for animal models for this situation, D (2mg/kg) and M (20 mg/kg) were administered perorally to male (n=5) and female (n=5) Cynomologus (C) and to male (n=3) and female (n=3) Rhesus (R) monkeys followed by a 24 h urine collection. In R monkeys, D/4-OH-D metabolic ratios were 0.23 ± 0.07 and M/4-OH-M metabolic ratios were 0.05 ± 0.03. By contrast, C monkeys exhibit marked sex differences of D/4-OH-D metabolic ratios (0.57 ± 0.94 and 2.94 ± 1.96 in males and females, respectively) and of M/4-OH-M metabolic ratios (0.77 ± 1.19 and 1.95 ± 1.29 in males and females respectively). Thus, the observed sex differences in D and M metabolism render C monkeys unsuitable for further studies relating to human pharmacogenetics and no D or M hydroxylation deficiency was found in R monkeys.

PHA 232

HALOTHANE DETERMINATION IN HUMAN SERUM BY HPLC

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The volatile anesthetic halothane (H) can be analyzed easily and accurately by GC in gaseous samples, however, the determination in serum is not as satisfactory. Therefore an HPLC methodology was developed allowing direct H analysis in serum. The HPLC apparatus consists of two pumps (A and B), an extraction- and an analytical-column, an injector, a 6-way valve and an UV detector. An aliquot of the serum is injected without any sample preparation directly onto the extraction column in a polar mobile phase. Under these conditions the polar constituents of the biological material such as proteins, aminoacids, electrolytes etc. pass through and are eluted to waste, whereas the lipophilic H is retained on the extraction column. After 2 min the valve is switched automatically, whereby the analytical mobile phase (pump A_1) backflushes the loaded content (H and other apolar constituents of the serum sample) from the extraction column on to the analytical column, where H is separated and detected UV photometrically at 230 nm with a sensitivity of about 1 µg/ml. The HPLC procedure provides an analytical variability of less than 2 %. The suitability and reliability of the method can be demonstrated by studying the time course of H in the serum of anesthetized patients.

PHA 233

THE GENETIC CONTROL OF CODEINE BIOACTIVATION

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We found that codeine and other narcotics are competitive inhibitors of (+)-bufuralol 1'-hydroxylation and dextromethorphan O-demethylation, two prototype reactions for assessment of polymorphic cytochrome $P\text{-}450_{DB}$ function. Codeine O-demethylation to morphine was therefore investigated in liver microsomes incubates from 6 organ transplantation donors. In vitro morphine production was blocked by quinidine (K_i 2 x 10^{-8}M), the highly specific inhibitor of $P\text{-}450_{DB}$. In 5 extensive metabolizers microsomes (5 x 10^{-4}M codeine) morphine production was 11.8 (SD 3.5), 5.4 (1.6) and 2.6 (0.6) nmol x mg P^{-1} x h^{-1} respectively in controls, in presence of 10^{-7} and 10^{-6}M quinidine, whereas corresponding production was stable at 1.8 nmol x mg P^{-1} x h^{-1} in 1 poor metabolizer. Correlation coefficient between dextromethorphan and codeine O-demethylations was 0.97 ($P < 0.05$). Preliminary in vivo results confirm that codeine biotransformation to morphine is dependent on the polymorphic monooxygenase known as cytochrome $P\text{-}450_{DB}$ and that poor metabolizers have a more than 10-fold reduction in morphine production.

PHA 234

ETHOLOGICAL COMPARISON OF DEPRESSED AND HEALTHY SUBJECTS

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An ethological comparison was made of the behaviour of 13 depressed (DSM III, outpatients), and 13 healthy subjects, matched for age and sex. During the first 6 minutes of a semi-structured interview,over 130 items of body postures, facial expression, gaze and speech parameters shown by the subjects were recorded. Depressed patients exhibited less smiles, were less assertive, and showed fewer forehead and eyebrow movements than did healthy subjects. The latter, glanced more often at the interviewer, showed less gaze aversion, more visual searching and generally more social signals than did depressed patients. Speech parameters also differed. Moderate correlations were found between ethological and conventional psychiatric assessment scales. These results show that as in animals, ethological methods can be applied to the study of human behaviour and may prove useful in psychiatry. They also suggest that non-verbal measures of social behaviour could be a useful marker of recovery in depressed individuals.

PHA 235

EVALUATION OF AN IN VIVO/IN VITRO SHORT-TERM CARCINOGENICITY TEST.

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An in vivo/in vitro approach was evaluated for quantitative investigation of the carcinogenic potency of two model test compounds: MNNG, a chemical which hydrolyses to active species at first contact with a tissue, and procarbazine (MIH) belonging to a group of chemicals which require a multistep metabolic activation. Fibroblasts from a rat granulation tissue (granuloma pouch) were exposed to various doses of the two compounds in vivo or in vitro. As a suitable endpoint in vitro for chemical transformation colony formation in the soft agar assay was chosen. MNNG exposure in vivo or in vitro showed a dose-related effect in the induced transformation frequency, which was significantly higher in the MNNG-exposed than in the untreated cells. The dose response curve obtained after in vivo exposure of the cells to procarbazine, or after its i.p. administration, revealed also a linear dose-related effect. As expected, no induced colony formation in soft agar was found after in vitro exposure of the cells to MIH. By this method the expression time for the detection of neoplastic phenotypes can be shortened to 4-6 weeks.

PHA 236

CA^{2+}-BINDING PROTEINS AS MARKERS FOR CHEMICALLY TRANSFORMED FIBROBLASTS.

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Fibroblasts from a rapidly proliferating granulation tissue of the rat were exposed in vivo (or in vitro) to various doses of the carcinogens MNNG or procarbazine (MIH). Normal and chemically transformed fibroblasts were extracted in 0.4mM EDTA, pH 7.5. Proteins were ^{14}C-labeled by reductive methylation and analysed by 2D PAGE for the presence of two Ca^{2+}-binding proteins, namely oncomodulin (M_r 12kD and pI 3.9) and a protein with M_r 12kD and pI 4.8. Untreated fibroblasts lack these proteins. Their early appearance found during chemical carcinogenesis suggests that they might be useful markers for preneoplastic phenotypes. Co-migration experiments have been performed to identify both tumor-associated proteins. In addition, $^{45}Ca^{2+}$-transblot electrophoresis confirmed their Ca^{2+}-binding capacity. Antibodies against the two proteins will be produced in order to quantify the inducible transformation frequency at the single cell level.

PHA 237

MODULATION OF CICLOSPORIN A TOXICITY IN CULTURED RAT HEPATOCYTES BY INDUCTION OF THE BIOTRANSFORMATION CAPACITY

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Ciclosporin A (CsA) is extensively metabolized in the liver by the cytochrome P-450 system. This study was designed to determine whether increases in the metabolic capacity could alter the concentration dependent cytotoxic effects of CsA in cultured rat hepatocytes. Pretreatment of rats with Aroclor 1254 increased the total cyt P-450 content in the liver by a factor of 2.7, including the specific CsA-metabolizing isozymes, as shown by increased CsA metabolism rates in cultured hepatocytes (38 % increase in metabolite formation after 17 hr using 10-6 M CsA). In these cells, the concentration dependent cytotoxic effects of CsA were less pronounced than the effects of CsA in cells from non-induced rats. These results demonstrate that an increased rate of CsA metabolism reduced CsA toxicity in vitro.

PHA 238

COMPUTER ANALYSIS OF THE ULTRASONIC VOCALIZATION IN RAT PUPS PRE- AND POSTNATALLY EXPOSED TO ETHANOL AND METHYLMERCURY

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The vocalizations of 5 to 15 day old rat pups, isolated in a glass chamber at a temperature of 20 C, were recorded during one minute by an ultrasonic microphone. The frequency of the amplified and filtered signal was converted to voltage and digitized at a sampling rate of one per 4 ms. A LSI-11 computer calculated the total number, and the duration, interval and frequency distributions of these cries. In a first experiment, rat dams were treated with ethanol (20% or 30% of their caloric intake) during the whole pregnancy. In a second experiment methylmercury was administered in the drinking water (1.5 or 5 mg per liter) during pregnancy and lactation. In both experiments the ultrasonic vocalizations showed strong dose effects. The total number of cries during subsequent sessions indicated a delayed development due to the treatment both with methylmercury and ethanol. Methylmercury in the highest dose also reduced the absolute number of cries. The ethanol treated animals showed a dose dependent shift of the frequency spectrum especially at day 7 and 9.

PHA 239

METABOLIC FATE AND COLONIC DNA DAMAGING ACTIVITY OF FOOD BORNE HETEROCYCLIC AMINES.

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There is an association between high meat consumption and colon carcinogenesis in man. The heterocyclic amines 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) and 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx) are two potent bacterial mutagens which are formed in cooked beef products and are multipotential carcinogens to CDF1 mice (Sugimura, Mutation Res. 150 [1985] 33-41; Ohgaki et al., Carcinogenesis 5, [1987] 665-668. Neither chemical led to tumour development of the large intestine, however, IQ induced tumour growth in both the large and small intestines of F344 rats. In our laboratory, OF1 and Ae/MS mice treated by gavage with IQ or MeIQx developed nuclear aberrations of the large intestine, indicating a potential for carcinogenesis. Metabolic studies in rats have shown that biliary metabolites of MeIQx are present as non-mutagenic, detoxified products (Turesky et al., submitted to Carcinogenesis). Present work in our laboratory using conventional and axenic mice is underway in order to examine the involvement that the gut flora have in the metabolic activation of these amines.

PHA 240

GENOTOXICITY OF SOME TRICYCLIC ANTIDEPRESSANTS IN DROSOPHILA

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Several tricyclic antidepressants were tested for genotoxicity using the Somatic Mutation And Recombination Test, SMART. Three day old larvae of Drosophila melanogaster trans-heterozygotic for two linked recessive wing hair mutants (multiple wing hairs and flare) were fed the test compounds in water mixed with a standard dry food. The larvae feed until pupation, ca. 48 hrs. When the adult flies emerge their wings are mounted on slides and examined for the presence of twinned and untwinned spots of mutant cells. The frequency of spots is compared to controls. At least one of the compounds, imipramine, proved genotoxic at concentrations above 1.0 mM while amitriptyline & protriptyline were not genotoxic at concentrations up to the highest tested (100 mM). Preliminary results indicate that these 3 compounds may reduce the spontaneous mutation rate at low concentrations. Results of combinations of tricyclics with a known mutagen (ENU) will be presented as well as results of tests with other compounds of similar structure.

PHA 241

QUANTITATIVE ANALYSIS OF THE ACTIVITY PATTERN OF RATS IN ACUTE TOXICITY TESTS

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The effects of acetanilide and phenylmercuric acetate on the activity pattern of rats were analyzed by passive infrared monitoring. This method provides a quantifiable activity measure of small laboratory animals in their home cage. Rats are housed individually in Macrolon® cages equipped with a passive infrared sensor. The detection area is adjusted to the dimensions of the cage. The sensors turn off for 1.5 ± 0.5 seconds when the animal moves. They are connected by an interface to an Epson HX-20 microcomputer which is programmed to check all sensors in one-second intervals. The activity measures are added and registered as 5-minute blocks. Acetanilide leads to an immediate, dose-dependent reduction of activity in all treated rats. The survivors showed normal activity pattern already on the day after treatment. Phenylmercuric acetate leads to a dose-dependent reduction of activity with maximum on the fifth day and lasting for 2 weeks.

PHA 242

INFLUENCE OF A TEMPORARY 30 % DIETARY RESTRICTION ON LIVER TUMOUR DEVELOPMENT AND ON SERUM INSULIN LEVELS IN MICE.

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A 30 % dietary restriction drastically lowers the rate of the development of liver tumours induced by diethylnitrosamine in mice. To further characterise this effect, the rate of tumour development was investigated in temporary restricted animals by measuring the glucose-6-phosphatase deficient foci incidence and sizes. When dietary restricted animals were fed an ad libitum regimen at 12 or 24 weeks of age, the tumour development was accelerated but was still slower than in continuously ad libitum fed animals. When ad libitum fed animals were dietary restricted at 12 or 24 weeks of age, the tumour development was considerably slowed down. Even some foci have completely regressed. Insulin is known to be a growth modulator of the hepatocytes. We measured the serum insulin levels after a glucose challenge in the different groups. Restricted animals had lower insulin levels than the ad libitum fed animals. A switch to an ad libitum feeding increased the insulin level whereas a switch to a restricted diet caused a decrease. These results sugges the involvement of insulin in the mecanism by which dietary restriction influences liver tumour development.

Cell and Molecular Biology (CMB)

CMB 243

A BINDING SITE FOR ALTERNATIVE FACTORS ON THE MOUSE ALBUMIN PROMOTER.:

Wuarin,J., Lichtsteiner, S., Mueller, C. and Schibler, U. Dépt de Biologie moléculaire, Uni Genève, CH-1211 Genève 4.

We have identified five distinct transcription factors binding to six specific sites within the mouse albumine gene. These DNA-binding proteins include CBP/EBP, NF-I, pseudo-NF-I, NF-Y, and HNFI (Lichtsteiner et al,Cell vol 51, 1987). At least some of the cis-acting DNA elements can be occupied by alternative factors. Thus site E has affinity for the ubiquitous factor NF-I and for the liver-specific factor pseudo-NF-I. Another more proximal site (C), that contains the motif CCAAT, can be filled with either the ubiquitous protein NF-Y or the liver-specific protein CBP/EBP. Interestingly, the binding of NF-Y on site C and the binding of CBP/EPB on site D is mutually exclusive. We have performed saturation point mutation of site C to determine which of the two factors (NF-Y or CBP/EBP) is activating albumin transcription. Indeed, some of the mutants have selectively lost their binding capacity for one or the other factor. These mutant templates will be tested for in vitro and in vivo transcription.

CMB 244

A STEMLOOP STRUCTURE IN THE 3' NONTRANSLATED REGION IS REQUIRED FOR IRON-DEPENDENT REGULATION OF CYTOPLASMIC TRANSFERRIN RECEPTOR mRNA

Müllner, E., Kühn, L.C.; ISREC, Genetics Unit, CH-1066 Epalinges

The expression of human transferrin receptor (hTR) and its mRNA is strongly induced by the iron chelator Desferrioxamine (Desf). We introduced a series of mutations into the 3' nontranslated region of a hTR cDNA expression vector and transfected these constructs into mouse L cells. Expression analysis of deletion mutants revealed 2 domains of less than 100b each and about 600b apart which are both essential for regulation by iron. Computer prediction of mRNA folding within this region indicated a stem loop structure. In vivo analysis of additional point mutations and deletions affecting this stem loop confirmed the importance of the secondary structure in regulation. By nuclear run on assays and by measuring the amount of hTR specific nuclear RNA in the presence or absence of Desf it could be shown that the regulation occurs posttranscriptionally in the cytoplasm. This finding was confirmed by measuring the half life of cytoplasmic hTR mRNA in the presence or absence of Desf, Actinomycin D and Cycloheximide. The 3' nontranslated region of hTR also conferred iron dependent regulation to a human HLA-A2 mRNA in vivo.

CMB 245

INTERFERENCE OF A PUTATIVE HUMAN TUMOR SUPPRESSOR GENE WITH H-RAS AND v-MOS INDUCED GENE EXPRESSION IN NIH 3T3 CELLS

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The transformed phenotype can be induced by the addition of glucocorticoid hormone to the culture medium in NIH 3T3 cells transfected with oncogene constructs under the control of a MMTV LTR (R. Jaggi et al., EMBO J., 5: 2609-2616, 1986). Cellular transformation by H-ras and v-mos is associated with morphological alterations, the ability to proliferate in soft agar and tumorigenicity in nude mice. We observed a repression of glucocorticoid hormone dependent transcription, a transient stimulation of c-myc gene expression, a constitutive increase of ODC mRNA and a mitogenic stimulus. Introduction of a putative tumor suppressor gene (R. Schäfer et al., Proc. Natl. Acad. Sci. USA, in press) prevents the hormonal induction of the transformed phenotype in NIH 3T3 cells containing a MMTV LTR v-mos or LTR H-ras construct. Data on the effect of the suppressor gene on the oncogene induced changes in endogenous gene expression will be presented.

CMB 246

REGULATION OF THE HUMAN UROKINASE-TYPE PLASMINOGEN ACTIVATOR GENE TRANSFECTED INTO MOUSE L CELLS.

Cajot JF[1], Schleuning WD[2], Medcalf RL[2], Testa JE[1], Mead H[3], Liebermann L[3], and Sordat S[1]. Swiss Institute for Experimental Cancer Research, 1066 Epalinges, Switzerland,1; Central Hematology Laboratory, University of Lausanne Medical School, 1011 Lausanne, Switzerland, 2; BIOGEN Res. Corp., Cambridge, MA 02142 , USA, 3.

A cosmid (cospUK0322) harbouring the complete human urokinase-type plasminogen activator (u-PA) gene isolated from a human genomic library was transfected into mouse L cells. Ten transfection clones were isolated and shown to express plasminogen activator (PA) activity. The secreted PA was shown to be identical to human pro-u-PA in enzymatic, electrophoretic and antigenic properties. Regulation of expression of the transfected gene was studied by treatment of the cells with various effectors. The most striking results were obtained with retinoic acid (2x10-6M: 7-fold stimulation) and dexamethasone (10-7M: 5 fold suppression). In addition, agonists of the adenylate cyclase dependent pathway such as dibutyryl cyclic AMP, cholera and pertussis toxins increased u-PA expression. All changes in enzyme activity were associated with comparable changes of u-PA mRNA steady state levels. u-PA is believed to play a role in extracellular matrix degradation in tumor invasion and metastasis. Reconstituted systems of the kind described here may allow the design of experiments that could address this question more directly.

CMB 247

NEGATIVE REGULATION OF IMMUNOGLOBULIN EXPRESSION

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When immunoglobulin (Ig) producing B cells are fused with fibroblasts, expression of immunoglobulins is selectively suppressed at the transcriptional level. We have shown that a rearranged Ig gene stably introduced into myeloma cells is also down regulated after fusion. To further define the DNA sequences within the Ig transcription unit which are involved in that down regulation, we have linked the IgH enhancer or the kappa light chain promoter to a B-globin test gene and introduced these constructs into myeloma cells. When these myeloma clones are fused with fibroblasts, B-globin expression ceases. Nuclear "run-ons" showed that this suppression is transcriptional.In control myeloma cells where the B-globin gene is on its own or driven by the SV40 enhancer, fusion with fibroblast cells does not lead to suppression of B-globin expression. Further experiments in vivo and vitro are in progress.

CMB 248

CONSTITUTIVE FACTORS BIND TO THE VIRUS RESPONSIVE ELEMENT OF THE HUMAN IFN-α1 GENE (VREα).

Nicholas J. MacDonald, Deborah Maguire, François Meyer and Charles Weissmann. Institut für Molekularbiologie I, Universität Zürich, CH-8093 Zürich.

Inducibility of the IFN-α1 gene is mediated by a 46-nucleotide promoter segment, the virus responsive element VRE(IFNα), which is located between positions -109 and -64 relative to the transcription start site. This sequence confers virus inducibility on a reporter gene when placed upstream of or within its promoter in either orientation. We have used electrophoretic band shift assay and DNase I footprinting to investigate the interactions of cellular factors with the VRE(IFNα). At least 4 retarded bands have been detected using nuclear extracts prepared from non-induced Namalwa cells. DNase I footprinting experiments reveal a single protected region between position -86 and -72 using either non-induced or virus-induced cell extracts. We are currently purifying these binding factors in order to further determine their role in the regulation of interferon gene expression.

CMB 249

ERYTHROID SPECIFIC ACTIVATION OF THE XENOPUS LAEVIS ADULT α - GLOBIN PROMOTOR IN TRANSIENT HETEROKARYONS

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1.5 kb of the 5' flanking region of the adult α-globin gene of X.laevis cloned in front of the CAT structural gene promote synthesis of CAT in transiently transfected X.laevis kidney cells. Fusion of transiently transfected kidney cells with erythroblasts isolated from anaemic frogs stimulates CAT expression 2-4 fold in the resulting transient heterokaryons. The stimulation is specific for the α-globin promotor and is obtained after fusion with erythroid cells but not with hepatocytes or kidney cells.

Stably transfected kidney cells contain drastically reduced CAT activity as compared with transiently transfected cells. However, fusion of stably transfected kidney cells with erythroblasts leads to a > 10 fold induction of CAT activity.

We conclude that erythroid specific transacting factors stimulate expression of CAT controlled by the adult α-globin promotor.

CMB 250

TETRAMERIC HEXANUCLEOTIDES THAT MEDIATE SILENCING, AND INDUCIBILITY BY VIRUS AND BY INTERFERON

Madan Chaturvedi, Hubert Hug, Marcel Wehrli, Nicholas MacDonald, Dietmar Kuhl, François Meyer, Markus Aebi and Charles Weissmann, Institut für Molekularbiologie I, Universität Zürich, CH-8093 Zürich

Tetramers of GAAAGT and GAAAGC (the monomers found in VREα, the IFN-α virus response element), and of GAAACT, GAAACG and GAAAGG (present in IRE(Mx), the IFN response element of the Mx gene), conferred virus inducibility on a reporter gene when placed between SV40 enhancer and TATA box. Tetrameric GAAAGT, GAAACT and GAAAGC in addition mediated IFN response and under non-induced conditions silenced potentiation of transcription by the SV40 enhancer. Tetrameric hexanucleotides contain 6 permutations in various spacings. Analysis of variants of $(GAAAGT)_4$ suggests that the sequence essential for virus induction is $GNAAGTN_{(5\text{-}8)}GNAAGTG$. Requirements for IFN induction and silencing are more restrictive. Induced or uninduced cell extracts gave a specific gel retardation pattern with sequences mediating silencing.

CMB 251

ANALYSIS OF THE TRANS-ACTIVATION AND DNA BINDING FUNCTIONS OF THE RAT GLUCOCORTICOID RECEPTOR.

Y.Severne, S.Wieland, M.Thali, I.Galli, O.Georgiev, W.Schaffner and S.Rusconi, Institute of Molecular Biology II, University of Zürich, 8093 Zürich.

The DNA binding and transcriptional activation functions of the glucocorticoid receptor (GR) coreside in a Cys-rich region of the protein (aa 440-525). A mutagenesis cassette with 13 additional restriction sites was created by silent mutations in the cDNA. Three major phenotypes emerge from the analysis of mutant GRin CV1 cells: permissive, semi- and non-permissive mutations. Moreover, chimaeric GR/GCN4 proteins were constructed by linkage of an additional DNA binding domain, derived from the yeast transcription activator GCN4, to a truncated GR. These bifunctional chimaeras can induce transcription via two distinct enhancer elements; the GRE or the yeast His3-UAS motifs. We study the effect of GR mutations in these chimaeras to assign a precise role to specific aminoacids. We also try to obtain revertants from the most interesting mutations.

CMB 252

THE PROMOTERS OF TISSUE-SPECIFIC AND DEVELOPMENTALLY REGULATED HISTONE GENES OF THE SEA URCHIN INTERACT WITH A NOVEL TRANSCRIPTION FACTOR

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Institut für Molekularbiologie II der Universität, Hönggerberg, CH-8093 Zürich

Nuclear extracts of sea urchin embryos contain a novel factor that binds to the -60 region of two late H2B-2 promoters between the octamer sequence and the TATA box, and to the -100 region of two late H2A-2 promoters. This factor has been operationally termed Late Histone Transcription Factor (LHTF). Competition experiments revealed an identical affinity of LH TF for all four binding sites, which exhibit surprisingly low sequence similarity.Deletion analysis of one of the late H2B-2 promoters showed that both the octamer element and the recognition sequence for LHTF are required for efficient in vitro transcription in the gastrula nuclear extract. LHTF is absent from sea urchin testis nuclear extracts and it does not interact with promoters of early and sperm histone genes. Moreover, LHTF does not bind to the promoter of a late H2B gene that is ubiquitously expressed in adult sea urchin, thus suggesting its possible role in both the developmental and tissue-specific regulation of a particular sub-family of late histone genes.

CMB 253

MOLECULAR BASIS FOR THE REGULATION OF MATING BEHAVIOUR OF FEMALE DROSOPHILA MELANOGASTER

Chen, P. S., Stumm-Zollinger, E., Aigaki, T., Balmer, J., Böhlen, P. and Bienz, M., Institute of Zoology and Institute of Biochemistry, University of Zürich, CH-8057 Zürich

We isolated a peptide from the male accessory glands of Drosophila melanogaster by HPLC. It consists of 36 amino acids and was fully sequenced. Injection of a physiological dose (3 pmole/fly) of this peptide into virgin females switched off sexual receptivity, induced extrusion of ovipositor and stimulated oviposition. cDNA was synthesized starting from poly(A)$^+$RNA of the accessory glands and cloned into M13mp8. Screening of the plaques with synthetic mixed oligonucleotide probes yielded 3 clones with positive signals. The inserts of these clones were sequenced by the dideoxy method and the cDNA was completed to the start codon by primed extension using again a synthetic oligonucleotide. The transcript has a total of 255 nucleotides. Northern analysis showed that expression of the transcript is male- and tissue-specific.

CMB 254

NEGATIVE REGULATION OF MOUSE MAMMARY TUMOR VIRUS IN NON-TARGET CELLS

Garcia, M., Vessaz, A, Billotte, J. and Diggelmann, H., ISREC, 1066-Epalinges

Epithelial cells of the mammary gland are not the only targets for the tumorigenicity of mouse mammary tumor virus (MMTV) and several observations suggest that it might also be involved in kidney-cell transformation. The analysis of the biological properties of MMTV-M (mammary origin) and MMTV-K (kidney origin) were investigated by measuring their relative transcriptional efficiencies in epithelial cells of mammary and kidney origin. S1 mapping analysis showed that MMTV-K was transcribed in both cell types whereas MMTV-M was efficiently expressed only in mammary gland cells. We then tested the hypothesis that a sequence element in the LTR possibly binding a trans-acting factor was exerting a negative regulatory effect on viral gene expression. The transcriptional potential of various plasmids containing the complete or parts of the LTR of MMTV-M or MMTV-K was determined after transfection of mammary and kidney cells. The results showed that sequences present in the LTR of MMTV-M repress the transcription from the viral promoter in kidney cells. We therefore conclude that the MMTV-LTR contains a negative regulatory element that prevents MMTV expression in non-target cells.

CMB 255

CELLULAR RNA SEQUENCES CAN SERVE AS LEADERS IN VACCINIA VIRUS LATE mRNAs.

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We have recently described the unusual structure of a vaccinia virus late mRNA (Bertholet et al., Cell 50, 153, 1987). These molecules are characterized by the presence of long leader RNAs indicating discontinuous transcription.
The mRNA of another late gene has now been analyzed. In one such RNA the protein-coding sequences were also preceded by a long leader sequence. In this case however, the leader consisted of the first 263 nucleotides of 18S ribosomal RNA which was fused to the viral sequences via a poly(A) tail. Screening of a cDNA library prepared from poly(A)-containing RNA isolated late in infection with a ribosomal probe showed that about 0.5% of all cDNA clones contained polyadenylated ribosomal RNA fragments. Most likely virus infection induces breakdown of ribosomes resulting in RNA fragments that are then polyadenylated by the viral poly(A) polymerase. Such molecules seem to be one of the sources from which leader sequences for the formation of mature late mRNAs are recruited.

CMB 256

CLONING OF A *XENOPUS* VITELLOGENIN FULL LENGTH cDNA IN AN EUCARYOTIC EXPRESSION VECTOR AND FUNCTIONAL ANALYSIS IN ESTROGEN-RESPONSIVE CELLS.

Nardelli Haefliger, D., Cayroll, C. and Wahli, W., Institut de Biologie Animale, UNI CH-1015 Lausanne

To characterize the sequences involved in the *in vivo* estrogen(E_2)-dependent stabilisation of vitellogenin (vit.)mRNA, a cDNA library was constructed using liver mRNA of an E_2-induced *Xenopus laevis* female. A clone coding for the entire sequence of the vit.B1 mRNA was identified. The expression of this mRNA driven by an heterologous E_2-independent promotor has been tested in MCF7 cells in the presence or absence of E_2. No difference in the vit.mRNA levels was observed in this cell line, which was previously used to characterize the E_2-responsive sequences that regulate vit.gene promotors. These results show that no E_2-inducible elements acting on the used heterologous promotor are contained in the exons of the vit.gene. Furthermore, in these cells E_2 did not stabilise vit.mRNA suggesting that tissue or species specific factors are involved in the liver cells. We are currently testing this hypothesis in primary chicken hepatocytes.

CMB 257

TRANSCRIPTIONAL REGULATORY ELEMENTS FOR PROGESTINS AND ANDROGENS IN THE MOUSE MAMMARY TUMOR VIRUS (MMTV) PROMOTER REGION.

Gowland, P. and Buetti, E., ISREC, 1066-Epalinges

Plasmids with various mutations in the hormone regulatory region of the MMTV long terminal repeat were introduced by transient or stable transfection into the T47D human mammary tumor cell line, which contains functional receptors for progestins and androgens, but not for glucocorticoids. Hormonal effects were detected in a quantitative S1 nuclease protection assay. Androgens gave about 10-fold lower inductions than progestins. In contrast to the results obtained with glucocorticoids, with progesterone the promoter-proximal region was most sensitive to mutations, while the distal region was barely sensitive. This was confirmed by co-transfecting an active gene of the glucocorticoid receptor into the same cells. A synthetic oligonucleotide of the distal region failed to restore full progesterone stimulation when inserted into a proximal region mutant, while it did for glucocorticoids. Therefore, despite the described coincidence of *in vitro* binding sites for the respective receptors, the contribution of proximal vs. distal elements are functionally distinct for the two hormones.

CMB 258

ANALYSIS OF cAMP-RESPONSIVE ENHANCER ELEMENTS IN THE HUMAN SUBSTANCE P GENE. A. LoMonico, A. Young, T. Bonner and H.-U. Affolter (sponsor: U. Karli), Brain Research Institute, Univ. of Zurich, 8029 Zurich, Switzerland, and NIH, Bethesda, MD, USA.
Fusion genes containing portions of the 5' flanking regions of the human substance P gene linked to the CAT reporter gene were introduced into RD-4 (human rhabdomyosarcoma) or AtT-20 (rat anterior pituitary tumor) cells and tested for responsiveness for forskolin, an activator of adenylate cyclase. These experiments showed that the human substance P gene is regulated by cAMP. Promotor deletion mutations along with analysis of the gene sequence revealed that at least 2 cAMP regulatory elements (CRE) are present within the 5' flanking region of this gene. An additional CRE was found to be located within exon 1. Individual analysis of CRE's in a reporter/competitor assay revealed that these enhancer sequences showed different affinities to a cellular factor(s) involved in cAMP mediated gene expression. Full activity of the promotor and precise initiation of RNA transcription appear, however, to be dependent upon the CRE located most distant from the TATA box.

CMB 259

REGULATION OF IL-2 RECEPTOR EXPRESSION BY IL-1: MOLECULAR ANALYSIS.
Plaetinck, G., Combe, M.C., Corthésy, P. and Nabholz, M., I.S.R.E.C. Genetics unit, CH-1066 Epalinges s/ Lausanne.
Previously we have shown that in a mouse x rat T cell hybrid, PC60, IL-2 receptors can be induced by IL-1 and IL-2 and that regulation occurs at the mRNA level. Induction of PC60 in the presence of cycloheximide shows that the effect of IL-1 is protein synthesis independent. Interestingly, by itself, cycloheximide can already induce IL-2Rα* mRNA. PC60 cells transfected by electroporation with the human IL-2Rα cDNA in a SV40 ex-pression vector were selected for stable expression of the human α chain. mRNA and protein levels of the transfected gene could still be strongly increased by IL-1 but not by IL-2. As it is unlikely that IL-1 regulates transcription from the SV40 promotor this predicts that the information for regulation by IL-1 is probably contained within the sequence of the IL-2α cDNA. Measurements of mRNA stability after treatment with Actinomycin D indicates that pre-incubation with IL-1 results in a twofold increase in the halflife of the mRNA. Because this is not enough to account for the over 10 fold increase in the steady state level observed, regulation by IL-1 must involve additional mechanisms.
*the mouse homologue of the human "Tac" gene.

CMB 260

IMMEDIATE-EARLY TRANSCRIPTS OF BOVINE HERPESVIRUS-1

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The genes of human herpesviruses are expressed in a temporal cascade, producing immediate-early (IE), early (E), and late (L) transcripts, which give rise to IE, E, and L-proteins. Permissive cells express IE-genes in the absence of de novo protein synthesis and can therefore be enriched in IE-transcripts by cycloheximide treatment. The IE-proteins are important regulators of the lytic infection cycle; some are required for activation of E- and L-genes and for autoregulation of IE-genes, and some have also been implied in latency. Much less is known about animal herpesviruses. Last year we showed that bovine herpesvirus-1 (BHV-1), an important pathogen in cattle, also exhibits a transcription-pattern in a temporal cascade. This report concerns detection and mapping of the BHV-1 IE-transcripts. Total RNA was extracted from BHV-1 infected cells incubated 0 to 3 hours post infection or treated with cycloheximide for 6 hours. This RNA was analyzed by northern-blot- and S1-mapping-experiments using probes from our cloned genome library of BHV-1 strain K22. We could identify at least 5 IE-transcripts, one at the left end of the genome and 4 clustered on the HindIII fragment C (map unit 0,73 - 0,85). In crosshybridizing experiments the 3,9 kb IE-transcript of BHV-1 (map unit 0,744 -0,781) gave a clear signal with a probe of the single IE-gene of pseudorabies virus. Analysis of a possible structural relationship between BHV-1 and herpes simplex virus-1 IE-transcripts and identification of transcripts during latent BHV-1 infection are in progress.

CMB 261

THE HUMAN ESTROGEN RECEPTOR hER CAN REGULATE TRANSFECTED VITELLOGENIN GENES COPIES BUT NOT THE ENDOGENOUS GENES IN A XENOPUS CELL LINE.
Seiler-Tuyns,A., Mérillat,A.M., and Wahli, W.
Institut de Biol. Animale, Dorigny, 1015 Lausanne
An estrogen responsive element (ERE) has been characterised in the 5' flanking region of the estrogen inducible Xenopus vitellogenin genes. Further understanding of the mechanisms regulating these genes requires the identification of other cis-acting elements if they exist as well as trans-acting regulatory factors.Transfection experiments into the Xenopus kidney cell line B3.2 show that hER acts as a regulatory trans-acting factor.Both hER and estrogen are necessary for the induction of vit-CAT chimeric genes and several mini genes.In these cells, induction of the minigenes tested is similar to that of the vit-CAT constructs, suggesting that sequences within the structural parts of the vitellogenin gene present in the minigenes do not contribute to the induction process.The experiments also indicate that factors other than hER are required for the induction of the endogenous gene copies of the B3.2 cells, since these genes remain silent in contrast to the transfected constructs.

CMB 262

IDENTIFICATION OF TWO TRANSCRIPTION SITES IN THE 5'-END FLANKING REGION OF THE *XENOPUS* VITELLOGENIN GENE A_1.
Batistuzzo de Medeiros,S.R.,Tremea, F.,ten Heggeler-Bordier,B.,Germond,J-E.,Seiler-Tuyns,A. and Wahli, W., Institut de Biologie Animale de l'Université, CH-1015 Lausanne
Analysis,*in vivo* and *in vitro*, of the expression of the vit.gene A_1 revealed two transcription initiation sites 1.8 kb apart.*In vivo*, both sites are estrogen-sensitive in liver but one site,i, is 50-times more active than the other,i_0.The strong i site is the one homologous to that used by the other *Xenopus* vit. genes.In contrast to the i site,there is no sequence homologous to the estrogen-responsive element (ERE) close to the i_0 site.To test if the ERE which controls the activity of the i site also can act on the i_0 site,several chimeric genes containing different portion of the A_1 5'-end region linked to the CAT reporter gene were constructed.Their transient expression was tested after transfection into the human estrogen sensitive MCF-7 and the *Xenopus* B3.2 kidney cell lines.Both initiation sites are used in these cells and their differential activity will be discussed.

CMB 263

ANALYSIS OF FRACTIONATED XENOPUS LIVER EXTRACTS BY IN VITRO TRANSCRIPTION, OOCYTES MICROINJECTION AND FOOTPRINTING ASSAYS

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In Xenopus laevis, the expression of the vitellogenin genes occurs exclusively in the female liver. In order to study the factors involved in this tissue-specific and hormone-regulated activation, we have prepared several liver protein extracts. These extracts contain all the components required to reconstitute estrogen-controlled in vitro transcription of the B1 vitellogenin promoter. We then separate the proteins on Heparin-Sepharose or Mono-Q columns and tested the fractions for their ability to transcribe the homologous vitellogenin promoter, or to induce the vitellogenin locus by microinjection into Xenopus oocytes where these genes are normally silent. The capacity of these crude and separated extracts to bind specific regions on the promoter is also presented .

CMB 264

CHARACTERIZATION OF THE CELL TYPE-SPECIFIC DETERMINANT IN THE GENOME OF MINUTE VIRUS OF MICE.
J.-Ph. Antonietti and B. Hirt, Swiss Institute for Experimental Cancer Research, 1066 Epalinges.
There exist two strains of minute virus of mice (MVM) that show different host cell specificities. The prototype strain MVM(p) grows in fibroblasts whereas the immunosuppressive variant MVM(i) grows in T-lymphocytes. In this study, we have mapped on the viral genome a cell type-specific determinant which permits growth in fibroblasts: it is located between 69 m.u. and 85 m.u. in a region coding for the viral capsid proteins. The DNA of MVM(p) does not replicate in lymphocytes. MVM(i) is unable to help MVM(p) to grow in lymphocytes; thus the determinant acts in a cis fashion. During a restrictive infection of lymphocytes with MVM(p) we did not detect viral mRNA. However, when the same cells were transfected with cloned DNA, both MVM(p) and MVM(i) DNAs were transcribed with the same efficiency from both promoters and the RNA was processed normally. Therefore, the specificity determinant is not a cell type-specific enhancer.

CMB 265

TRANS-ACTIVATION OF MINUTE VIRUS OF MICE P38 PROMOTOR BY THE NS-1 NONCAPSID PROTEIN.
Ch. Doerig and J.-Ph. Antonietti, Swiss Institut for Experimental Cancer Research, 1066 Epalinges.
The genome of the autonomous parvovirus MVMi is organised in two overlapping transcription units: the genes coding for the two non-structural proteins (NS-1 and NS-2) are transcribed from a promoter (P4) located at map unit 4, whereas the promoter for the capsid protein genes (P38) lies at map unit 38. We studied the effect of NS-1 and NS-2 on the activity of the P38 promoter in vivo. P38 activity was tested in two ways: on one hand using a plasmid containing the chloramphenicol acetyl transferase gene under the control of the P38 promoter, and on the other hand by measuring mRNA concentration after transfection. By site-directed mutagenesis we constructed clones encoding only NS-1 or only NS-2. The activity of the P38 promoter was measured in cells transfected with constructs containing the genes for one or both viral NS proteins. We found first that the P38 promoter of MVMi is activated in trans by a viral gene product. Second we could identify the viral protein responsible for the trans-activation as being NS-1.

CMB 266

A TRANSCRIPTION FACTOR FROM SV40 CHROMOSOMES WHICH ACTIVATES THE VIRAL LATE PROMOTER. P. Beard and H. Bruggmann, Swiss Institute for Experimental Cancer Research, 1066 Epalinges.

We extracted, from SV40 chromosomes, a factor which activates transcription from the SV40 late promoter. We then looked at binding of this factor to the origin-promoter region of SV40 DNA, and compared it with the binding of transcription factors from HeLa cells.

The late activating factor was separated from DNA and RNA and partially purified using hydroxylapatite. On addition to transcription reactions with SV40 DNA and components from HeLa cell nuclei, it activated transcription from the major late start site at position 325.

In DNase I footprinting experiments a partially purified late activating factor preparation protected a DNA region spanning SV40 nucleotides 250-300, which lie between the repeated 72-bp. enhancer and the major late RNA start site. Proteins from neither HeLa cells, non-permissive mouse cells nor uninfected CV-1 cells gave an equivalent footprint at this position.

CMB 267

TWO ENHANCER ELEMENTS UPSTREAM AND DOWNSTREAM FROM THE PSEUDORABIES IMMEDIATE EARLY GENE: ANALYSIS OF CIS- AND TRANS-EFFECTS.

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The PRV IE gene is flanked by enhancer elements E3 and E4 that were isolated by virtue of their ability to complement enhancerless SV40. Enhancer activity was determined with constructions containing the chloramphenicol acetyltransferase (CAT) gene: E3 placed in front of the SV40 early promoter resulted in 2.6 fold stimulation. The PRV IE promoter by itself had a 6.6 fold higher activity than the SV40 promoter. The promoter activity of PRV could be enhanced by placing E3 either upstream or downstream of the CAT gene. The activity of E3 was abolished by separating it from the promoter/CAT gene with two restriction enzyme cuts, thus demonstrating cis-activity. The combination of PRV E3 and IE promoter was nearly as active as that of the SV40 enhancer and promoter (the prototype pSV2CAT). Surprisingly E4, while clearly active in the SV40 system, seemed to act negatively on the PRV promoter. By cotransfecting plasmids carrying the PRV IE gene or the trans-inducing factor (αTIF) of HSV-1 together with the CAT-plasmids, we could show that the PRV IE gene exerts a stimulatory effect on the SV40 promoter and an inhibitory effect on its own promoter (autorepression), whereas αTIF acts in the opposite way. This autorepression may perhaps explain the apparent lack of an enhancer effect on IE gene function measured by CAT or S1 nuclease analysis. Taken together, our results help to understand the regulatory network operating in the early stages of PRV infection.

CMB 268

REGULATION OF MOUSE MAMMARY TUMOR VIRUS (MMTV) TRANSCRIPTION BY GLUCOCORTICOIDS

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Our previous mutagenesis studies have delimited the distal glucocorticoid regulatory element of MMTV to 10 base pairs between -181 and -172 from the RNA start site. Although a proximal element and a nuclear factor-1 binding site are also required for maximal stimulation of transcription, the distal element provides the largest contribution to the response and was studied in more detail. The decanucleotide of the distal element was inserted in different defective mutants, and the plasmids tested by transfection assays and S1 mapping. This "minimal element" was able to restore the glucocorticoid response when inserted in the mutated proximal site in either orientation, or when inserted in a large distal site deletion in the reverse orientation, but not when present in its natural orientation: it had no effect, even in multiple copies, in larger deletions of the proximal element or of the whole glucocorticoid regulatory region. These recombinants are being tested for their ability to bind nuclear proteins in DNase I footprinting experiments.

CMB 269

INTRON RECOGNITION IN PLANTS: 3' SPLICE SITE SELECTION DIFFERS IN PLANTS AND ANIMALS,
Goodall G., Wiebauer K. and Filipowicz W., Friedrich Miescher Institut, CH-4002 Basel

We have compared the splicing of pre-mRNA in plants and animals by transfecting the soybean leghemoglobin and human β-globin genes into both plant protoplasts and HeLa cells. Analysis of the splicing patterns obtained indicates that plants and animals differ in the mechanism of 3' splice site selection. A survey of animal and plant gene sequences indicated that the polypyrimidine tracts present at the 3' end of animal introns are not present in plant introns. Many plant introns, however, are AT-rich throughout the entire intron. To test the significance of the AT bias in plant introns we have chemically synthesized a hypothetical plant gene of mostly random sequence, but incorporating 5' and 3' splice site concensus sequences flanking an AT-rich "intron", embedded in a GC-rich sequence. This gene is efficiently spliced at the predicted sites in plant protoplasts. Using a series of unique restriction sites within the synthetic sequence, we have made a series of modifications to test the contribution of various sequence elements to intron recognition.

CMB 270

Discontinuous transcription of the chloroplast psaA gene in C. reinhardtii.

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The chloroplast *psaA* gene of the green unicellular alga *Chlamydomonas reinhardtii* encodes one of the photosystem I reaction center polypeptides and consists of three exons that are widely scattered over the circular chloroplast genome. Exon 1 and exon 2 are oriented in opposite directions. Numerous nuclear and chloroplast mutants are specifically affected in *psaA* mRNA maturation and can be grouped into three phenotypic classes. Class A mutants are able to splice the transcripts of exon 1 and exon 2, but are unable to join the transcripts of exon 2 and exon 3. Class B mutants accumulate three independent transcripts of the three exons and are deficient in both joining steps. Class C mutants, which are able to splice the transcripts of exon 2 and exon 3, are unable to join the transcripts of exon 1 and exon 2. The phenotype of class B indicates that the three exons are transcribed independently as precursors which are normally assembled *in trans*. The occurence of class A and class C mutants implies that the splicing reactions can occur in either order. The primary transcripts of exon 2 extend far beyond the coding regions and include *psbD*, the gene encoding the D2 polypeptide of photosystem II.

CMB 271

REGULATION OF MOUSE HISTONE GENE EXPRESSION BY RNA 3' PROCESSING: CHARACTERIZATION OF U7 snRNA.

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The efficiency of histone RNA 3' processing plays an important role in the control of histone mRNA levels during the transition from quiescent to proliferating cells. One of the two identified components of the processing apparatus, a heat-labile activity, is directly involved in this regulation, whereas the second component, snRNPs of the Sm serotype, seems to play a constitutive role. It is known from studies on sea urchins that the rare U7 snRNA basepairs with a highly conserved processing signal on the histone pre-mRNA. By using DNA oligonucleotides spanning the vertebrate processing signal, we have been able to synthesize and sequence cDNA for mouse U7 RNA. This RNA is immunoprecipitable with antibodies of the Sm serotype and shares several structural features with its sea urchin equivalent. We are presently engaged in studying the structures of the U7 gene(s) and of the corresponding snRNPs.

CMB 272

THE MOLECULAR BASIS FOR rRNA-DEPENDENT STREPTOMYCIN RESISTANCE IN E. COLI

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The antibiotic streptomycin (Sm) interacts with the small subunit of the ribosome and causes misreading of the message, abolishing normal protein synthesis. We have shown that Sm resistance can be due to a single C to T transition in E. coli 16S rRNA gene at position 912 (Montandon et al., EMBO J. 5:3705-3708, 1986). Positions G888 and C912 are complementary in 16S rRNA and represent the last base pair of helix 27. To determine whether disruption of helix 27 correlates with Sm resistance, we have mutagenized G888 by site-directed mutagenesis. We will report on these experiments and others aimed to study the importance of neighboring positions in Sm resistance.

CMB 273

EXPRESSION OF ENDOGENOUS AND EXOGENOUS mRNA CODING FOR Na,K-ATPase SUBUNITS IN XENOPUS OOCYTES

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The minimal functional Na,K-ATPase unit consists of an α-β subunit(Sα,Sβ) protomer. We have studied the biosynthesis and intracellular processing of Sα and Sβ in Xenopus oocytes (stage VI). Sα, but not Sβ, could be immunoprecipitated from microsomal(P) and plasma membrane(PM) fractions of metabolically labelled oocytes indicating that only Sα but not Sβ is synthesized in fully grown oocytes. As assessed by controlled trypsinolysis, the newly synthesized Sα was highly trypsin sensitive and not able to perform cation-dependent conformational changes. Sβ could only be detected on immunoblots in PM and by immunoprecipitation in surface labelled oocytes. On the other hand, injection of size-fractionated mRNA coding for Sβ of frog and toad kidney cells led to the expression of fully glycosylated Sβ in P and PM suggesting that lack or modification of endogenous Sβ mRNA is responsible for the lack of oocyte Sβ synthesis. In view of these data, the Xenopus oocyte might become a valuable tool to study the importance of Sα-Sβ assembly for the functional maturation of Na,K-ATPase.

CMB 274

DOES SEMLIKI FOREST VIRUS (SFV) CAPSID(C)-PROTEIN ACT AS A PLEIOTROPIC REGULATOR OF HOST CELLULAR PROTEIN SYNTHESIS?

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It has been suggested that the C-protein is the component responsible for the shut-off of host protein synthesis in SFV infected cells (Van Steeg et al., 1984, Eur.J.Biochem. 138:473-478). As shown elsewhere (see poster Elgizoli et al.) low concentrations of microinjected C-protein (10^3 to 10^4 molecules per cell) resulted in a stimulation of cellular protein synthesis. Here we demonstrate that high concentrations (10^5 to 10^6 molecules per cell) led to a significant repression (up to 50%) of protein biosynthesis. Similar to induction, repression lasted only for about 2 h after delivery of C-protein. By microinjecting large numbers of C-protein molecules we observed their rapid association with the nucleus, reaching a maximum already 30 min after delivery.

CMB 275

INDUCTION OF CELLULAR PROTEIN SYNTHESIS BY LOW CONCENTRATIONS OF SEMLIKI FOREST VIRUS (SFV) CAPSID(C)-PROTEIN

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Microinjection of highly purified SFV C-protein allows to study its presumed interactions in the regulation of translation of cellular mRNAs in the absence of other viral functions such as transcription, translation, and assembly. We have shown previously that C-protein molecules (M_r 33 kD) are efficiently incorporated into electroporated cells (Michel et al, Experientia, in press). Here we demonstrate that low concentrations of C-protein (10^3-10^4 molecules per cell) introduced by electroporation, liposome- and red-cell-ghost-mediated delivery induced host cellular protein synthesis up to 2.5 fold. The induction observed in 3 different cell lines (CV1, P3X63Ag8, and GPBind4) was time dependent and lasted for about 2h after microinjection.

CMB 276

AN ESSENTIAL YEAST PROTEIN HOMOLOGOUS TO THE MAMMALIAN TRANSLATION INITIATION FACTOR eIF-4A CAN, WHEN OVEREXPRESSED, SUPPRESS A MITOCHONDRIAL MISSENSE MUTATION.

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During our investigations in nucleo-mitochondrial interactions we have isolated two genes from S.cerevisiae, TIF1 and TIF2. The two genes code for two identical proteins of 395 aa. The two potential yeast proteins are highly homologous to the translation initiation factor eIF-4A from mouse (Nielsen et al. NAR13, 6867). In the yeast protein 257 aminoacids are identical and 62 aminoacids are similar to the mouse protein resulting in 81% identity/similarity.

Genetic analysis of the two genes indicates that one or the other gene has to be active. Inactivation of TIF1 or TIF2 by gene disruption experiments does not lead to a mutant phenotype. However, cells carrying the two inactivated genomic genes can only be obtained if the function of TIF1 is provided on a plasmid. It is thus clear that one or the other gene has to be active for survival of the cell. This is in agreement with the idea that the genes TIF1 and TIF2 code for a translation initiation factor.

The TIF genes have originally been isolated as suppressor genes for a missense mutation in the mitochondrial coxIII gene. This suppression is only observed if the genes TIF1 or TIF2 are present on multicopy plasmids stressing the importance of a gene dosage effect. The possible roles of the TIF genes in mitochondrial biogenesis are investigated.

CMB 277

STRUCTURAL STUDIES ON CHLOROPHYLL A/C LIGHT HARVESTING COMPLEX FROM THE CRYPTOMONAD CRYPTOMONAS MACULATA: PARTIAL AMINO ACID SEQUENCES

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The chl a/c light harvesting complex of the cryptomonad Cryptomonas maculata (showing an apparent molecular weight of 80 000 Da) was cleaved in 70% formic acid at the Asp-Pro bonds. The resulting large fragments were further separated by reversed phase HPLC using a system with a gradient of (A) 6 M HCOOH, 0.1 % TFA and (B) 4 M HCOOH, Acetonitrile/ Isopropanol 3:1, 0.1% TFA (according to Tarr). The peptides were analyzed on a Applied Biosystem Microsequencer ("Gas-phase" sequencer). The amino acid sequences show sequence homologies to light-harvesting chlorophyll a/b protein complexes of plants. A amino acid sequence homology to the LHC II complex of pea (Bürgi et al., BBA 890,346-351,1987) was found:

PEA LHC: ...P E A F A E L K V K E L K N G R L A M F . . .
chl a/c: ...P E G F A ? A Q L K E V K N G R L A M I . . .

CMB 278

CHARACTERIZATION OF THE *ANTENNAPEDIA* GENE PRODUCT OF *DROSOPHILA MELANOGASTER*

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The homeobox-containing *Antennapedia (Antp)* gene of *Drosophila* is required for the normal differentiation of thoracic segments during embryonic development and metamorphosis. To characterize the *Antp* gene product, we have synthesized the *Antp* protein in *Drosophila* embryos containing a P-element-transformed *Antp* gene fused to a heat-inducible *hsp70* promoter. In these embryos the protein is overexpressed approximately 50 fold after two hours of heatshock at 37°C. We have raised rabbit antibodies against an *E.coli*-synthesized, truncated *Antp* protein. On Western blots the wildtype *Antp* protein and the heatshock *Antp* protein have the same apparent molecular weight of about 57 kd. Gel filtration and native gel electrophoresis experiments suggest that the protein exists in solution as a complex of 220-250 kd.Two-dimensional gel electrophoresis resolves the protein into a row of spots which range in pH from 6.5 to 7.5, indicating that the protein is covalently modified in *Drosophila* embryos. We are investigating the nature of these post-translational modifications and their role in altering the function of the protein.

CMB 279

LIGNIN PEROXIDASE GENE FAMILY

Reiser,J.*, Kälin,M.*, Walther,I.*, Fritsche,B.*, Casimir,J.+, Saloheimo,M.+, Teeri,T.+, Suter,F.*, Knowles,J.+, Leisola,M.+, and Fiechter,A.*, Institut für Biotechnologie ETH-Hönggerberg, 8093 Zürich* and Biotechnical Laboratory, VTT, SF 02150 Espoo, Finland+

The white-rot basidiomycete Phanerochaete chrysosporium produces a number of extracellular peroxidases which appear to be important for lignin degradation. With a view towards overproducing these enzymes in heterologous hosts we have isolated a number of lignin peroxidase-related cDNA clones using antibody and oligonucleotide probes. Hybridization experiments revealed a number of distinct DNA segments in Southern blots indicating that the lignin peroxidase genes are reiterated in the P. chrysosporium genome. One DNA clone was isolated from a P. chrysosporium chromosomal gene bank. It harbours two lignin peroxidase-related gene copies contained in a DNA segment of about 20 kb. The DNA sequences of some of the cloned segments indicate that there are a number of partially homologous lignin peroxidase genes in P. chrysosporium.

CMB 280

ISOLATION AND PARTIAL AMINO ACID SEQUENCES OF THE 89KD-ANCHOR POLYPEPTIDE OF PHYCOBILISOMES FROM MASTIGOCLADUS LAMINOSUS

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Phycobilisomes of the cyanobacterium Mastigocladus laminosus are light-harvesting pigment-protein complexes which are attached to the photosynthetic membrane via a 89kD anchor polypeptide. This protein has a covalently bound phycocyanobilin chromophore that transfers light energy to PSII in the membrane. The 89kD polypeptide was isolated from intact phycobilisomes by gel filtration in 0.1% SDS. It was chemically fragmented with CNBr and the resulting peptides were isolated by gel filtration and reverse phase chromatography. The following amino acid sequences were obtained from automated Edman degradation on a gas-phase microsequencer:

N-terminus: (S)VKA(S)G(G)...
a) DVFSGQVPEYFRRPDLESKLRNGEIKVREFVR(D)LA(S)XXIY(R)K(S)FY...
b) DVLITEFKAPTPSNKVRQRPSGDQQ(G)L(L)LPQIYFNAAQR(W)QKE...
c) LNTQEVAEAFGEDTVPVNRFPTLPAAN(F)PNTQKLYNQLXKQP...
d) KEFVRRLGKSPLY(K)(R)QFYEP...
a-d) CNBr-fragments

CMB 281

Aldolase Activity associated with a Plasmodium falciparum antigen.

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The 41 Kd protein, expressed at the schizont and merozoite blood stage of P. falciparum, is a candidate for the development of a malaria vaccine : it is recognized by immune human sera and it was shown to induce a protective immune response in monkeys. The gene encoding p41 has been cloned from genomic and cDNA libraries. In particular a cDNA clone of 1250bp has been identified and subcloned in the pUC18 plasmid (pGF2). E. coli transformed with this plasmid express a recombinant protein recognized by serum of rabbits immunised with 41 Kd purified from P. falciparum and by anti 41 Kd Monoclonal Abs. The DNA sequence shows a significant homology between p41 and fructose 1-6 disphosphate aldolase (human and rabbit). Along this line, we observed that RBCs infected with P. falciparum have more than 10 times higher aldolase activity than normal RBCs and that this activity is inhibited by rabbit anti 41 Kd serum. Consistently with these results E. coli harboring pGF2 (and expressiong p41) show increased levels of aldolase, also sensitive to anti 41 Kd rabbit serum.

CMB 282

PURIFICATION OF RNA PHOSPHATE CYCLASE FROM HeLa CELLS

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We have purified to homogeneity RNA 3'-terminal phosphate cyclase from HeLa cell extracts. In the presence of Mg^{2+} and ATP, this enzyme catalyzes the conversion of a 3'-phosphate group into the 2',3'-cyclic phosphodiester at the 3'end of RNA, through formation of a covalent cyclase-AMP intermediate. GTP, CTP and UTP (but not dATP or ADP) can also act as cofactors in the cyclisation reaction, although less efficiently; consistent with this, the enzyme can be covalently labelled with the four (α-^{32}P)NTPs. The cyclase is highly resistant to proteases but very sensitive to heat inactivation and to SH group reagents. The physiological role of this enzyme is unknown. Since reactions catalyzed by eukaryotic RNA ligases require 2,3'-cyclic phosphates at the end of RNA, it is possible that RNA cyclase is involved in the generation of cyclic ends required for RNA ligation or splicing. We are studying the mechanism of reaction and substrate specificity of RNA cyclase in order to gain some insight into its physiological function.

CMB 283

NEURITE-PROMOTING ACTIVITY OF LAMININ- AND TENASCIN-LIKE MOLECULES FROM LEECH EXTRACELLULAR MATRIX

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Identified leech neurons grow cell-type specific neurite patterns in defined medium when plated on cell-free ganglion capsule extracellular matrix (ECM). Neurite-promoting activity has been solubilized from ECM with EDTA and purified by gel chromatography. Upon rotary shadowing, active fractions were found to contain cross-shaped macromolecules resembling vertebrate laminin and six-armed structures similar to vertebrate tenascin. The laminin-like leech molecule was purified by affinity chromatography to a monoclonal antibody, and shown to consist of subunits of M_r = 220,000 and 340,000. Attachment of leech neurons to this protein was sufficient to initiate rapid sprouting in defined medium. In contrast, leech neurons did not grow neurites on vertebrate laminin, and vertebrate neurons failed to sprout on the leech protein. The tenascin-like leech protein from leech ECM might be a second neurite-promoting component since vertebrate tenascin (which is related to the CNS proteins J1 and cytotactin) mediates the sprouting by vertebrate neurons.
Supported by grants to J. Nicholls from S.N.F. and US Navy.

CMB 284

FUNCTIONAL ANALYSIS OF *DROSOPHILA* HOMEOBOXES

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Mutations in the segmenation *gene* fushi tarazu (ftz) *cause* embryonic lethality: homozygous *ftz*$^-$ embryos die as larvae possessing half the number of segments of wildtype embryos. We showed earlier, that one copy of the *ftz* $^+$ gene with 6.1 kb 5'-flanking sequences, including cis-acting controlling elements, suffices to rescue homozygous *ftz*$^-$ embryos. To study the function of the homeobox, we replaced a *ftz* homeobox fragment by the homologous part of two other homeoboxes differing in their amino acid sequences, namely *Scr* and 99B. The fusion homeoboxes still encode the putative helix-turn-helix motif thought to be important in protein-DNA interaction. The two new fusion *ftz* genes were transformed back into the flies. Their function was tested in homozygous *ftz*$^-$ background. The *Scr* replacement, leaving the α3 recognition helix intact but changing five amino acids upstream, can rescue *ftz*$^-$ embryos, whereas the 99B replacement cannot. The 99B homeobox is only distantly related to the *ftz* homeobox,and the second amino acid in the putative recognition helix (Arg-Thr)is changed. These results support the model that homeoboxes can be grouped in families that contact the same regulatory elements.

CMB 285

IDENTIFICATION OF THE MEMBRANE-INTERACTING POLYPEPTIDE OF THE BROMELAIN-SOLUBILIZED ECTODOMAIN OF INFLUENZA VIRUS HEMAGGLUTININ BY HYDROPHOBIC PHOTOLABELING.

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The bromelain-solubilized ectodomain of influenza virus hemagglutinin (BHA) has been shown previously to bind to membranes at low pH. To investigate the molecular basis of this interaction, which most likely reflects an important step in the fusion process, we applied the technique of hydrophobic photolabeling using two different photoactivatable probes. Photolysis of BHA in the presence of liposomes containing [^{125}I]TID, a small, lipid-soluble,photosensitive molecule or [^{3}H]PTPC/11, a new phospholipid-analogue, whose distribution is confined to the lipid core of a membrane, resulted in label incorporation predominantly in the BHA2 subunit in a pH-dependent manner. Protein chemical degradation is now being used to identify this (those) polypeptide segment(s) which is (are) involved in the membrane interaction. Initial results strongly suggest that the interaction with liposomes is mediated (solely) by the N-terminal , highly conserved region.

CMB 286

Mx PROTEIN EXPRESSION DURING RECOVERY OF MICE FROM INFLUENZA VIRUS INFECTION

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Recovery of mice from influenza virus infection is influenced by the interferon- regulated Mx gene on chrs. 16. Mx$^+$ alleles encode the 72 kD nuclear Mx protein which protects cells against influenza viruses. Most laboratory mouse strains carry non- functional Mx$^-$ alleles and are highly susceptible to lethal infection with influenza viruses. Thus, a hepatotropic variant of influenza A virus grows rapidly in the livers of Mx$^-$ mice, causing fulminant generalized liver necrosis and death. In contrast, virus multiplication in Mx$^+$ mice is restricted to a few focal lesions which are self-limiting. By double immunofluorescence staining, we could demonstrate that these lesions contain viral antigen in the center and that the non-infected cells surrounding each lesion contain Mx protein, indicating that local Mx gene expression at the site of infection is essential for survival.

CMB 287

THE NUCLEOCAPSID OF BERNE VIRUS: ISOLATION AND STRUCTURE

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Berne virus (BEV) is a member of a recently discovered group of enveloped animal viruses for which a family status has been proposed ("Toroviridae"). Toroviruses are characterized by an unusual morphology and a distinct polypeptide pattern. Treatment of BEV with detergents and enzymes resulted a) in a better insight into the virion architecture and b) in the assignement of the main structural virion protein to a defined virus substructure. a) Purified BEV particles adsorbed onto formvar-coated EM-grids were degraded by different detergents and enzymes and negatively stained. A tubular nucleocapsid with a transverse striation was visualized indicating a helical symmetry of the viral core. b) Triton X-100 or NP40 (final concentration 1% and 0,7%, respectively) were added to a purified BEV preparation. Viral substructures thus obtained were separated by centrifugation on a rate zonal gradient (sucrose 5-20%). Analysis of the fractions by PAGE and immunoblotting showed 3 of the 4 structural proteins accumulated near the meniscus, whereas the fourth, a phosphorylated 20kD protein, penetrated into the gradient. A fraction containing only this protein revealed typical nucleocapsid structures when observed in the electron microscope.

CMB 288

LEVELS OF THE Ca^{2+}-BINDING PARVALBUMIN CORRELATE WITH TESTOSTERONE SYNTHETIC ACTIVITY IN LEYDIG CELLS OF RAT TESTIS

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Previous studies indicated that the Ca^{2+}-binding protein parvalbumin may be involved in the production of testosterone in Leydig cells of rat testis, a process which is highly dependent on calcium. This view is strongly supported by the following experiments:
Rats were hypophysectomized at two stages of development: (a) before puberty (postnatal day 23) when mostly immature Leydig cells were present which did not synthesize testosterone, and (b) at midpuberty (postnatal days 30-35) when mature hormone synthesizing Leydig cells were present. Hypophysectomy resulted in a loss of hormone production and simultaneously in a disappearance of parvalbumin-immunoreactivity. Repletion of the animals by FSH and LH, which turned on the synthesis of testosterone, was paralled by the reappearance of parvalbumin in the Leydig cells. These and further experiments strongly suggest an involvement of parvalbumin in the Ca^{2+}-dependent regulation of testosterone synthesis in rat Leydig cells.

CMB 289

MILK FEVER AND CALCIUM-BINDING PROTEINS

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Milk fever is characterized by a hypocalcemia and a reduction of tissue Ca^{2+}-concentrations. We tried to obtain more information of the altered homeostatic mechanisms which control the rate of supply of Ca^{2+} in response to the sudden increase in demand of Ca^{2+} which occurs at calving. Ca^{2+}-binding proteins are involved in the regulation of Ca^{2+}-concentrations, -mobilization, -transport and activation of a number of enzymes. We measured the levels of Ca^{2+}-binding proteins in body fluids and several tissues of normal and diseased cows. Differences in the concentration of some "putative" Ca^{2+}-binding proteins were found in the milk. In the salvary gland calbindin D-28K was reduced in diseased animals. Results will be discussed in relation to future therapies.

CMB 290

Ca^{2+}ACTIVATED NEUTRAL PROTEASE FROM ALLOMYCES ARBUSCULA

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A Ca^{2+}-activated neutral protease, having functional relationship to the animal enzyme, although distinct in many physical properties, has been purified and characterized recently from an aquatic fungus Allomyces arbuscula. The enzyme,a homodimer of 40kDa, is highly glycosylated and activated by millimolar Ca^{2+}. It binds to plasmamembranes in response to Ca^{2+}. Plasmamembranes and phospholipids, particularly phosphatidylethanolamine increases the sensitivity of the enzyme to Ca^{2+}. The Ca^{2+}binding region is resolved as a distinct 22 kDa fragment by trypsin digestion of the enzyme. Cyanogen bromide and N-chorosuccinimide recognises 7 and 5 cleavage sites respectively, representing as many methionine and tryptophane residues.

CMB 291

IMMUNOPURIFICATION OF HIGHLY SPECIFIC ANTIBODIES TO CALMODULIN FROM NEUROSPORA CRASSA.

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Highly specific antibodies against calmodulin from Neurospora crassa were arisen in rabbits. These antibodies were immunopurified by chromatography on Neurospora calmodulin-Sepharose, and had a titer for purified calmodulin from Neurospora crassa and bovine testis of 80 ng/ml and 2.5 µg/ml respectively. By immunoblot, as little as 8 ng of pure Neurospora calmodulin could be detected, and the antibody revealed calmodulin in crude homogenates from Neurospora crassa and Aspergillus nidulans while in homogenates from Allomyces arbuscula, Saccharomyces cerevisiae, Dictyostelium discoideum and bovine testis calmodulin remained undetected.

CMB 292

ANALYSIS OF THE MECHANISM UNDERLYING THE MEMBRANE FUSION INDUCED BY THE SPIKE PROTEINS OF SEMLIKI FOREST VIRUS (SFV) UPON LOW pH TREATMENT.

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It is known that SFV can induce membrane fusion at low pH. In order to characterize the mechanism by which the spikes may induce fusion, we have performed tryptic digestion of virions in the presence and absence of hydrophobic ligands. In the absence of ligands both E1 and E2 were trypsin sensitive. In the presence of ligands, E2 remained trypsin sensitive whereas 15% of E1 became trypsin resistant. After low pH treatment in the presence of ligands, E2 was again degraded but all of E1 became completely trypsin resistant. These results show that initial binding to the ligand is essential for the unfolding of the fusogenic conformation of E1. The marked increase in hydrophobicity of E1 probably favours its insertion into the lipid bilayers of the opposing membrane to initiate the fusion.

CMB 293

SITE DIRECTED MUTAGENESIS OF ROUS SARCOMA VIRUS PROTEINS p12 AND p10

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The amino acid sequence of Rous Sarcoma Virus (RSV) nucleic acid binding gag protein p12 contains two conserved regions called Cys-His boxes. We have used site directed mutagenesis to delete precisely either the first, or the second, or both Cys-His boxes. The mutant DNAs were analyzed in a transient transfection assay. We found that : 1) the deletion of either the first or the second box has no effect on the amount of RNA packaged in the particles but results in incomplete 70S dimer formation; 2) the deletion of the two boxes inhibits viral RNA packaging; 3) the deletion of the first but not of the second box inhibits the covalent binding of p12 to RNA upon UV irradiation and suppresses any detectable infectivity; the mutant which has only the first box remains slightly infectious. We have then prepared point mutants in every one of the amino acid of the Cys-His box and we are in the process of analyzing them. We have also constructed mutants of protein p10, the fifth gag protein whose function is unknown. Results with both p12 and p10 mutants will be presented.

CMB 294

INTERACTION BETWEEN HUMAN ESTROGEN RECEPTORS AND THE ESTROGEN RESPONSIVE ELEMENT

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The human estrogen receptor (hER) is a trans-acting regulatory protein composed of putative DNA-binding and steroid binding domains and two other regions influencing transcriptional activity. We have prepared nuclear extracts from Xenopus oocytes which had been injected with a human estrogen receptor expression vector (HEO) and show here that they can form complexes on the estrogen responsive element (ERE) of a Xenopus vitellogenin gene, as visualized by electron microscopy. In order to assess the properties of the hER domains, we have also analyzed several hER mutants. Mutants lacking the DNA binding domain are not able to induce complex formation, while those without the N terminal domain or the hormone binding domain do form complexes. The ability of the oocyte to synthesize functional hERs is demonstrated by the trans-activation of an estrogen-responsive CAT gene.

CMB 295

A PROTEASE WHICH RECOGNIZES DOUBLE BUT NOT SONGLE ARG-SITES

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Starting with the lysate of chromaffin granules from bovine adrenal medulla, we elaborated an enzyme assay based on the HPLC detection of the degradation of BAM 12P and the formation of metenkephalin and metenkephalin-Arg in function of time. The processing activity was purified by chromatography on Mono Q, on Phenyl Superose and on Superose 12. After a 1200 fold purification, the enzyme fraction was homogenous on disc gel electrophoresis (apparent M=70 000). The degradation rate of BAM 12P by the purified protease had its maximum at pH 5.6, in agreement with the internal pH of the granules. The specificity of the enzyme was determined by analysis of the degradation fragments of BAM 12P which showed that cleavage occurred at the double but not at the single Arg-site of BAM 12P. The behaviour of the enzyme towards specific inhibitors showed that it was as acid thiol protease different from serine proteases and from lysosomal cathepsin B. The specificity of cleavage of the enzyme, its localization in the chromaffin granules and its pH-optimum make it a serious candidate for a maturation enzyme in vivo.

CMB 296

SITE-DIRECTED MUTAGENESIS OF IGF II C-DNA

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The insulin-like growth factors (IGF I and IGF II) are polypeptides with structural and functional homologies to (pro)-insulin. While it is established that IGF I acts via the type I receptor as mediator of growth hormone on cell and body growth, the physiological function of IGF II via the type II receptor is unknown. One approach to elucidate its role is to restrict the affinity of IGF II to the type II receptor by site-directed mutagenesis. The target of mutation should be part of the binding site to the type I receptor postulated from computer-assisted modeling. The mutation of Tyr-B25 to Glu seemed most promising. This mutant was constructed by oligonucleotide-directed mutagenesis (Eckstein method), using a 465bp IGF II c-DNA inserted in MI3mpI8. The mutagenic primer was a I8mer oligonucleotide containing two mismatches. This mutated IGF II c-DNA will be expressed in E.Coli and receptor-binding studies with purified type I and type II receptor will be done.

CMB 297

ISOLATION OF HUMAN PLACENTAL ALKALINE PHOSPHATASE VARIANTS IN TUMOUR CELLS OF THE GERM LINE AND OF SOMATIC ORIGIN

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Placental alkaline phosphatase (PLAP) is expressed in placenta and embryonal germ cells of higher primates. Two variants of human PLAP have been described (termed PLAP and PLAP-like) which exhibit identical M_r of 64 kD on SDS-PAGE but are inhibited by different amino acids. PLAP or PLAP-like are found in trace amounts in a variety of normal tissues but their production is markedly enhanced in tumour cells. Thus PLAP and PLAP-like can be considered oncodevelopmental markers. We have isolated PLAP and PLAP-like from normal and tumour tissues of germinal and somatic origin by immunopurification with rabbit anti-PLAP-IgG coupled to Protein A-Sepharose. PLAP and PLAP-like were then separated by isoelectric focusing (IEF) as they show different pI, pI(PLAP)≈4.7; pI(PLAP-like)≈4.2, and further characterized by their inhibition pattern in the presence of leucine or phenylalanine. PLAP-like was enhanced in tumour cells of somatic origin (K 562, HeLa), whereas PLAP was markedly enhanced in germ line tumour cells (seminoma). We have thus demonstrated an accurate method for detecting and separating both isoforms and provided evidence for their differential expression in tumour and normal tissues of both somatic and germinal origin. (supported by the Roche Research Foundation)

CMB 298

NUCLEOCYTOPLASMIC SHUTTLING OF NUCLEOLAR ANTIGENS

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Many intracellular proteins are classified as either "nuclear" or "cytoplasmic", but developmentally timed and/or hormonally controlled shifts in the distribution of individual proteins have been documented. To study the dynamics of nucleocytoplasmic interactions, we have developed two complementary approaches: These are based on examining, firstly, the migration of "nuclear" proteins in chick-mouse interspecies heterokarions, and, secondly, the fate of cytoplasmically injected antibodies directed against "nuclear" proteins. Based on our results we conclude, that two non-ribosomal nucleolar proteins (90kd and 40kd, respectively) shuttle rapidly between nucleus and cytoplasm, even though analyses of steady state distributions indicate a predominantly nuclear location. We propose, firstly, that many "nuclear" proteins may constantly shuttle between nucleus and cytoplasm, and, secondly, that protein shuttling may play an important role in nucleocytoplasmic communication.

CMB 299

DIFFERENTIAL INTRACELLULAR TARGETING OF TWO FORMS OF PLASMINOGEN ACTIVATOR INHIBITOR 2

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A number of human and murine cell types synthesize PAI-2, a high affinity inhibitor of plasminogen activators, which is a member of the serpin class of antiproteases. We have shown that PAI-2 exists in two topologically and biochemically distinct forms: a) a glycosylated, secreted form, which, following subcellular fractionation, is also found in a membrane-enclosed compartment of Golgi-enriched fractions; b) a cytoplasmic form, which is not detectably glycosylated. Both forms are functionally and immunologically equivalent. By Northern blot hybridization and by RNase mapping we detect only one species of PAI-2 mRNA. We conclude that this mRNA codes for both forms of PAI-2, and are currently investigating the mechanism whereby a single mRNA can generate both a cytoplasmic and a secreted protein.

CMB 300

DOES IN VITRO TRANSLATED POLYOMA VIRUS MIDDLE T ANTIGEN INTERACT WITH DOG PANCREAS MICROSOMES?

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Different mutants of polyoma virus middle T antigen (mT) were translated in a wheat germ cell-free translation system. Furthermore, the association of these proteins with dog pancreas microsomes (DPM) was studied. Only fusion proteins containing the signal sequence and the N-terminus of influenza virus hemagglutinin (HA) fused to mT insert into the lumen of DPM, become trypsin-resistant and are glycosylated. A mutant of the fusion protein that lacks the hydrophobic membrane anchor of mT is still inserted and glycosylated but is not associated with DPM membranes. Wild type mT and truncated forms, which lack the membrane anchor, do not associate with DPM as reflected by their trypsin sensitivity and the lack of glycosylation. In agreement with earlier in vivo studies we suggest that wild type mT inserts directly into the plasma membrane after synthesis on free ribosomes.

CMB 301

INTRACELLULAR TRANSPORT OF MICROVILLAR PROTEINS: IS THERE A DIRECT PATHWAY FROM THE GOLGI APPARATUS TO LYSOSOMES?

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Immunocytochemical studies have suggested that a fraction of newly synthesized brush border hydrolases may directly be transported from the Golgi to lysosomes by the crinophagic pathway. We have used subcellular fractionation to test this hypothesis in the intestinal epithelial cell line Caco-2. A method was developed for the purification of lysosomes which resulted in a 24-fold relative enrichment of the marker enzyme glucosaminidase. Cross-contamination with other organelles was minimal. Lysosomes were found to comprize intact brush border hydrolases. The profile of appearance and disappearance of these enzymes in lysosomes is compatible with a transport along the crinophagic pathway.

CMB 302

A ROLE FOR THE CYTOPLASMIC DOMAIN IN TRANSFERRIN RECEPTOR SORTING AND COATED PIT FORMATION DURING ENDOCYTOSIS

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The 65 amino acid cytoplasmic domain of transferrin receptor (TR) is essential for endocytosis of this protein (Rothenberger, S., B.J. Iacopetta, and L.C. Kühn, 1987, Cell, 49:423-431). We have investigated by electron microscopy the surface distribution of wild type and cytoplasmic deletion mutant human TR expressed in transfected L cells. Approximately 15% of wild type TR was concentrated in coated membrane structures, regardless of the level of expression. In contrast, only 2% of deletion mutant TR was present in coated areas of the membrane. Cell lines expressing more than 3 million wild type TR per cell were found to contain 3-4 fold more coated pits than non-transfected Ltk$^-$ cells. No increase was observed in a cell line expressing similarly high levels of cytoplasmic deletion mutant TR. These results suggest that the cytoplasmic domain plays an active role in sorting and endocytosis of TR by providing an assembly site for coated pit formation.

CMB 303

EFFECT OF NOCODAZOLE ON THE TRANSPORT OF PROTEINS TO THE APICAL AND BASOLATERAL CELL SURFACE IN THE INTESTINAL EPITHELIAL CELL LINE Caco-2

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A cell surface protease assay was developed to study the selective appearance of newly synthesized proteins at the apical or basolateral membrane of Caco-2 cells which were grown to confluency on nitrocellulose filters. In this system the microtubule-disrupting drug nocodazole had no effect on protein delivery to the basolateral surface but it reduced transport to the apical surface and lead to a missorting of apical proteins to the basolateral membrane. These results suggest that intact microtubules are essential for apical but not basolateral protein delivery in Caco-2 cells.

CMB 304

CONGENITAL SUCRASE-ISOMALTASE DEFICIENCY (CSID) IN MAN. EVIDENCE FOR DIFFERENT MUTATIONS INTERFERING WITH THE INTRACELLULAR TRANSPORT OF SUCRASE-ISOMALTASE

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CSID was studied at the protein and subcellular level in 8 patients by means of monoclonal antibodies to sucrase-isomaltase (SI). All CSID patients expressed high-mannose SI but its maturation and intracellular transport was blocked at different stages along the biosynthetic pathway. Three phenotypes were found: one in which SI accumulated in the ER, one in which SI was arrested and degraded in the Golgi and one in which enzymatically altered SI was transported to the brush border but was also missorted to other organelles. These results suggest that minor alterations, probably point mutations, lead to transport-incompetent and functionally altered SI in CSID.

CMB 305

ROLE OF DIMERIZATION IN THE INTRACELLULAR TRANSPORT OF INTESTINAL BRUSH BORDER HYDROLASES

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It was postulated that oligomerization may be a prerequisite for the exit of newly synthesized proteins from the ER. This hypothesis was tested for brush border hydrolases, known to be dimers in most species, by using chemical cross-linking and sucrose gradient centrifugation. In Caco-2 cells dipeptidylpeptidase IV (DPP IV) was found to dimerize immediately after complex glycosylation while sucrase-isomaltase stayed as a monomer. Dimerization of DPP IV was prevented by CCCP and monensin but did not depend on terminal glycosylation of N-linked carbohydrates as assessed by inhibition of mannosidase I. These data suggest that dimerization of DPP IV occurs in the Golgi or later. We conclude that dimerization cannot be a general prerequisite for protein transport from the ER to the Golgi.

CMB 306

VISUALIZATION OF VIRAL SURFACE ANTIGENS BY VIDEO MICROSCOPY

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Monolayers of Vero cells infected with Respiratory Syncytial virus were used as targets for the reaction of antiviral antibodies and their visualization with immunogold and immunfluorescence techniques. Observation of labeled specimens with bright field or with differential interference contrast microscopy in conjunction with digital image processing for contrast enhacement, background subtraction and averaging allowed the detection and characterization of discrete antibody-gold (10-20 nm) complexes bound to the viral or cellular surface. The suitability of this technology was further exploited for the observation of immunoreactions in real time and thereby represents a novel approach for the characterization of surface antigens with a molecular probe.

CMB 307

GENETIC APPROACH TO IDENTIFY TRANS-ACTING FACTORS

Kikinis,Z., Hofstetter,P., Altus,M.S., Nagamine,Y.

In cultured pig kidney epithelial cells, LLC-PK1, the urokinase-type plasminogen activator (uPA) gene is activated by treatment with a peptide hormone, calcitonin. Calcitonin acts through the cAMP-dependent protein kinase.To identify the components mediating action of calcitonin, we prepared mutant cells.
A uPA-gpt hybrid gene, which includes 5kb of the 5'flanking region of the uPA gene and the coding region of the E.coli XGPRT gene, was constructed and transfected into LLC-PK1 cells.A stable transformant cell line, A1, in which the induction of the uPA-gpt hybrid gene and of the resident uPA gene was controlled by calcitonin, was obtained. Then,A1 cells were mutagenized with EMS and screened for constitutive expression of the uPA-gpt hybrid gene. We obtained 10 mutant cell lines that have constitutively elevated concentration of gpt mRNA and uPA mRNA. We infer that these clones have mutations affecting the activity of trans-acting factors that recognize both the uPA-gpt hybrid and resident uPA genes. Further characterization of these mutant cells will be reported.

CMB 308

A NOVEL VIEW ON SEMLIKI FOREST VIRUS-INDUCED CELL-CELL FUSION OF BABY HAMSTER KIDNEY CELLS

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The initiation of Semliki Forest virus-(SFV)-induced cell-cell fusion from within of baby hamster kidney (BHK) cells differs from other cells (e.g. Aedes albopictus cells). In contrast to such cells it is mandatory for BHK cells to be brought back to a neutral pH after a short exposure to mildly acidic pH (below 6.2) for fusion to occur. This phenomenon can be explained by a sudden drop of the intracellular pH observed after clamping the extracellular pH below 6.2 - the pH required to trigger fusion - and by the thereby implied increased expenditure of ATP. Since the fusion from within of SFV-infected cells is energy dependent (Kempf et al., Arch Virol 95, 111-122, 1987; Kempf et al., Arch. Virol. 95, 283-289, 1987) the fusion process is blocked due to the ATP depletion.

CMB 309

CAN VIRAL ENVELOPE PROTEINS ACT AS OR INDUCE PROTON CHANNELS?

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The mechanism of the process leading to cell-cell fusion induced by enveloped viruses at a mildly acidic pH is as yet unknown. In this report we demonstrate that the fusion events induced by three viruses of different families, namely Semliki Forest (togavirus), vesicular stomatitis (rhabdovirus) and influenza (orthomyxovirus), share common features. In all three systems a sudden drop of the intracellular pH - below the critical extra-cellular pH required to trigger "fusion from within" (FFWI) - is observed. This influx of protons is specific and not due to a general leakiness of the plasma membrane, and therefore might be caused by the opening of a proton channel.

CMB 310

GANGLIOSIDE'S EFFECT ON THE METHYLTRANSFERASE ACTIVITY OF MICROSOMES FROM NORMAL AND CANCER HUMAN BREAST TISSUES.

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The effect of monosialogangliosides GM1,GM2,GM3 on the methyl transferase (PMTase-I) which catalyzes the transmethylation of phospholipids was investigated in microsomal membranes prepared from human non-neoplastic and from carcinoma breast tissues.The response of the PMTase-I was characteristic of the ganglioside type and concentration and of the tissue source. In non-neoplastic membranes, GM1 and GM2 exerced a maximal increase of the PMTase-I at 10-9 and 10-8 M respectively.On the contrary, in microsomal membranes from cancer tissues, the elevated basal activity was still inhibited in presence of 10-9 M GM1 reaching a 70% of inhibition at 5.10-4 M.The PMTase-I was reduced at lesser extent by GM2 with a 50% inhibition at 6.10-5 M, while GM3 did not exerce any effect although highly expressed on these membranes. It is suggested that sialogangliosides alone and or in conjonction with cholesterol might indirectly affect the methyltransferase associated with microsomes by changes in the organization of membrane phospholipids.

CMB 311

CHARACTERIZATION OF NUCLEAR ENVELOPE-ASSOCIATED NON-LAMIN PROTEINS

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To elucidate the architecture of the nuclear envelope, monoclonal antibodies were raised against fractionated chicken embryonic nuclei and screened for recognition of nuclear envelope-associated proteins. Three antibodies, termed U5, P1 and R7, were characterized in detail: Based on molecular weights (250kDa, 82kDa, and 55/54kDa, respectively) and on the results of cell-fractionation studies, the three corresponding antigens were readily distinguished from lamin proteins. Immunofluorescent staining showed that all three antigens were located at the nuclear envelope. However, whereas the R7 antigen showed a rather uniform distribution, labeling with U5 and P1 antibodies revealed strikingly punctate patterns, suggesting an association of the latter antigens with nuclear pore complexes. After mitosis, P1 and R7 antigens became incorporated into the reassembling nucleus much earlier than U5 antigen or lamin proteins. Most remarkably, immunofluorescent analyses of cryosections and immunoblotting experiments indicate that these envelope-associated antigens may be expressed according to tissue-specific patterns.

CMB 312

GLUCOSE EFFECTS ON OSCILLATIONS OF CYTOSOLIC CALCIUM IN PITUITARY CELLS (GH_3B_6)

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Glucose is a secretagogue for insulin; its metabolism influences cytosolic calcium, $[Ca^{2+}]_i$, in pancreatic islet beta cells. We have investigated, whether glucose has also direct effects on rat pituitary GH_3B_6 cells. Regulation of $[Ca^{2+}]_i$ in these cells is characterized by oscillations linked to spontaneous action potentials. TRH alters $[Ca^{2+}]_i$ in a biphasic manner: the first phase is a rapid and short-lived elevation of $[Ca^{2+}]_i$; in the second phase, TRH causes oscillations of $[Ca^{2+}]_i$, or enhances the frequency of spontaneous oscillations. In order to appreciate possible effects of glucose, GH_3B_6 cells were maintained for 18 hours in a balanced salt solution without nutrients. After such a treatment, spontaneous oscillations as well as the response to TRH were found unchanged. Addition of glucose (2.8 mM) to starved cells induced an arrest of the spontaneous $[Ca^{2+}]_i$ oscillations. Furthermore, glucose shortened the second phase of the TRH response. It is concluded that glucose may exert a direct modulatory influence on pituitary cell activation.

CMB 313

CORRELATION BETWEEN PROTEIN KINASE C ACTIVITY AND BIOLOGICAL RESPONSE IN ANGIOTENSIN II- AND PHORBOL ESTER-STIMULATED ADRENAL GLOMERULOSA AND AORTIC SMOOTH MUSCLE CELLS.

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The distribution of protein kinase C (PKC) activity between cytosolic and membrane fractions was determined in isolated bovine adrenal glomerulosa cells and in cultured rat aortic smooth muscle cells, stimulated with angiotensin II (Ang II) or/and with phorbol-12-myristate-13-acetate (PMA) a substitute for diacylglycerol to activate PKC. In adrenal glomerulosa cells Ang II caused a redistribution of PKC activity from the cytosolic to the membranous form, whereas in aortic smooth muscle cells Ang II induced an increase of both forms. In aortic smooth muscle cells incubation with 100 nM PMA for 20 minutes suppressed the cytosolic, but increased the membranous PKC activity, whereas in adrenal glomerulosa cells the same treatment with PMA strongly inhibited both forms of PKC activity. Stimulation of aortic smooth muscle cells with PMA elicited a dose-dependent (1 nM - 1 mM) prostacyclin production, and treatment with 10 nM PMA potentiated the Ang II-induced prostacyclin production. In contrast, incubation of adrenal glomerulosa cells with 100 nM PMA had no effect on basal aldosterone production, but strongly inhibited Ang II-induced aldosterone production.
Our results suggest that an increase of the membranous but not the cytosolic PKC activity represents an early step in the post-receptor activation following Ang II stimulation.

CMB 314

STRUCTURE OF A COMPONENT OF A PUTATIVE MEMBRANE PROTEIN COMPLEX MEDIATING CELL-CELL ADHESION IN DROSOPHILA

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One of the important questions in morphogenesis concerns the identification of cell surface molecules responsible for specific cell-cell interaction and intercellular signaling. We have partially purified a glycoprotein complex from a membrane fraction of third instar larvae which mediates cell-cell adhesion as assayed in a cell aggregation system we developed from Drosophila. Using an antiserum raised against the partially purified adhesion activity, we isolated three cDNAs which were all derived from the same gene. Their longest open reading frame encodes a protein of 954 amino acids, putatively representing the major component of the complex mediating cell adhesion, a 120 kd glycoprotein. The protein of the isolated gene exhibits several features characteristic for extracellular signal transducing proteins.

CMB 315

A SPECIFIC ASSOCIATION OF INTEGRIN AND TALIN ARISES *IN VIVO* AFTER PHORBOL ESTER TREATMENT OF PERIPHERAL BLOOD LYMPHOCYTES.

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The integrin receptors are a superfamily of heterodimeric, transmembrane glycoproteins that function in cell-cell or cell-matrix interactions. Members of the superfamily include the integrin complex in chicken, the fibronectin receptor on human osteosarcoma cells, the glycoprotein complex II_b/III_a on human platelets and the LFA-1 complex on human lymphocytes. Integrins have also been implicated in forming attachments to actin filaments thereby serving as transmembrane linkers between its extracellular ligands and the cytoskeleton. These attachments are thought to involve one or more intervening cytoskeletal proteins linked to integrin. In order to study possible physiologically relevant associations of integrin with cytoskeletal proteins, the integrin molecules on the surfaces of intact chicken peripheral blood lymphocytes were collected into caps by crosslinking with specific antibodies, and the capped cells were examined by double immunofluorescence techniques to determine whether particular cytoskeletal proteins were associated with the integrin caps. With resting lymphocytes the capping of integrin did not result in any detectable redistribution of either talin, vinculin, or α-actinin. However, antibody-induced capping of integrin with phorbol ester (TPA)-treated lymphocytes resulted in the association of talin, but not of vinculin or α-actinin with the integrin caps. These results suggest that a specific association of integrin and talin arises *in vivo* after phorbol ester treatment of peripheral blood lymphocytes. It indicates that there are *dynamic* membrane-cytoskeletal interactions that are *metabolically* regulated. My EMBO longterm fellowship is acknowledged.

CMB 316

MODIFICATION OF α-ACTININ IN *Dictyostelium discoideum*

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The cytoskeletal protein α-actinin of *Dictyostelium* solubilized in Triton X-114 partitions mainly into the aqueous, but also into the detergent phase in an approximate ratio of 25:1. This indicates the presence of a major hydrophilic and a minor amphiphilic form of the protein. The two forms are indistinguishable in size upon SDS-PAGE.

In vivo labelling of *Dictyostelium* cells with ^{14}C acetate, tritiated palmitate or myristate did not reveal any modification of α-actinin. However, inorganic ^{32}P appears to label the hydrophilic form of α-actinin.

The activated *ras* oncogene is known to produce modifications in the shape of mammalian cells. Another tumor promoting agent, PMA, induces release of α-actinin from cytoskeleton. We are thus investigating whether α-actinin phosphorylation is regulated by a pathway involving the oncogene *ras* and protein kinase C.

CMB 317

TUBULIN AND β-TUBULIN mRNA CHANGES DURING DEVELOPMENT OF NEUROSPORA CRASSA

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We are investigating the role of the microtubule cytoskeleton during germination and growth of N. crassa. We used the cloned β-tubulin gene of N. crassa as a probe in Northern blot hybridization to follow the appearance of the specific mRNA. β-tubulin mRNA was absent in dormant macroconidia and was detectable within 1h of incubation at 37°C in nutrient medium. The microtubule inhibitor benomyl had no effect on the appearance of the β-tubulin mRNA.
In crude and partially purified cell extracts two α-tubulins and one β-tubulin were revealed with monoclonal antibodies against mouse (Amersham) or yeast tubulins (Kilmartin et al. 1982). In dormant macroconidia, we detected only low levels of tubulins. These increased during germination to reach a maximum after 15h (exponential growth phase) and decreased after 48h (stationary phase). Our results suggest that tubulins of N. crassa are developmentally regulated.

CMB 318

HIGH YIELD PURIFICATION OF NEUROFILAMENTS (NF) AND THEIR CONSTITUENT PROTEINS NF200K,NF150K AND NF70K

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The purification of NF triplet proteins from bovine spinal cords without the axonal floating technique resulted in a 10-fold increase of the amount of purified proteins. Homogenization and centrifugation in 0.8M sucrose resulted in a pellet containing NFs, GFAP and various copurified cytoskeletal proteins (MAPs, fodrin, vimentin and actin). The NF proteins were separated by hydroxylapatite (HTP) chromatography in 8M urea. Elution with 0.1M-0.14M phosph. buffer removed GFAP and other contaminants whereas NF200K, NF150K and NF70K were eluted with 0.3M phosph.. Consecutive purification of the NF constituent proteins were performed by ion-exchange chromatography (DEAE and HTP) and by electroelution from polyacrylamide gels. Various monoclonal antibodies directed against these purified proteins bound to common epitopes of the alpha-helical rod domain of all three NF proteins or bound to the variable domains in the head- and tailpieces.

CMB 319

ACTIN ISOFORMS AND INTERMEDIATE FILAMENT COMPOSITION OF HUMAN MYOFIBROBLASTS

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The myofibroblast combines the ultrastructural and biological features of fibroblasts and smooth muscle cells. We have examined by immunofluorescence the distribution of α-smooth muscle actin and desmin, two cytoskeletal proteins present in smooth muscle cells, in myofibroblasts of different wounds and fibromatoses. α-smooth muscle actin and desmin were never found in myofibroblasts from normal granulation tissue and scars; they were present, constantly but in varying proportions, in myofibroblasts of hypertrophic scars and fibromatoses. Thus, in pathological wound healing and in fibromatoses myofibroblasts acquire biochemical markers of smooth muscle but they retain fibroblastic phenotypic features in normal wound healing. This finding may be of importance to the understanding of the mechanisms of wound contraction and of fibromatosis histogenesis; it may be of prognostic value in situations susceptible to evolve toward contracture.

CMB 320

CONSERVATION OF THE PAIRED DOMAIN IN HUMANS

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Our gene network concept predicts that the genes of any organism consist of network-specific domains. Therefore, if we find in a particular organism two domains to occur in genes of the same network and if these two domains are specific for this network, we expect to find these two domains in the same gene network and hence to be associated with each other in genes of this network also in other organisms. This prediction was tested for the paired and homeo domains of humans. Six different regions have been isolated from a human genomic library that hybridize with the paired domain at low stringency. Two of these human paired domains have been sequenced. They show a high degree of homology with the paired domains of Drosophila: only 22 of 116 amino acids are altered of which 12 represent conservative amino acid changes. Interestingly, after hybridization with two different types of homeo domains (prd, Ubx), four of the six human genomic clones exhibit signals in an identical set of DNA fragments for both probes. Therefore, it appears probable that also in human genes combinations of the paired and homeo domain occur.

Physiology (PHY)

PHY 321

DISTORTION OF A RADIAL MAZE: THE IMPORTANCE OF DECLIVITY AS A CUE.

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Rats'spatial ability was tested in an 8-arm radial maze with two of these turned into parallel arms. This distortion slowed down learning considerably. In order to further analyse this problem and the use of declivity to solve it, one of the parallel arms was tilted at 15 degrees from the horizontal plane. Indeed, groups with a tilted parallel learnt the maze faster than controls. In addition, the influence of the frequency of such a cue was tested by tilting two more arms. The number of tilted arms induced no difference in efficiency. Interestingly, even when maze patrolling was efficient, one parallel arm was visited last more often than by chance. Nevertheless, when the spatial position of the parallel arms was changed, rats transitorily ended their visiting sequences in the area of the previously parallel arms. This was probably due to a motor response related to the spatial environnement. In conclusion, a possible distortion of motor strategies caused by parallel arms needs to be further investigated, nevertheless, rats were apparently capable of using a small vertical angle as a visual or kinesthetic cue and this improved their foraging efficiency.

PHY 322

ILLUMINATION OF THE DRONE RETINA CAN CAUSE IONIC CHANGES IN THE GLIA IN THE ABSENCE OF ELEVATED EXTRACELLULAR [K^+].

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Increased extracellular K^+ concentration (K_o) resulting from light stimulation of photoreceptors in superfused slices of drone retina causes the glia to depolarize and also to take up K^+. We report that if illumination, over a wide range of intensities, was prolonged, then K_o fell back to or below its dark level within 10 min, as did the glial membrane potential. This return of K_o to its dark level was much more rapid than the time of equilibration with the superfusate, but can be explained by uptake of K^+ into the photoreceptors, from where it diffuses down the axons and out of the retina. Unlike K_o, intracellular glial [K^+], measured with an ion-selective microelectrode, remained elevated throughout the illumination. This result implies that uptake of K^+ by the glial cells can be stimulated by some unidentified signal that is not a rise in K_o.

PHY 323

COMPUTER ASSISTED NEUROPHYSIOLOGY: A GLOBAL APPROACH TO SINGLE UNIT STUDIES

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A general software framework has been developed for the analysis of single unit experiments in the cat auditory thalamus. Two data sets are obtained after such an experiment: histological sections and spike trains recordings. The analysis of the histology consists on one hand on digitalized serial sections suitable for 2D and 3D reconstructions and on the other hand on the standardized coordinates attributed to each single unit. Time related statistics and raster displays are computed from the spike trains data. The result of all of these analysis is introduced in a relational data base allowing the user to obtain graphic output linking together functional properties and anatomy and to extract data for further statistical analysises. An interactive neuronal network simulator can be used to check models of the activity in local circuits. The transfer of information among the packages is done with ASCII files. A color movie is presented illustrating most of the features of our system.

PHY 324

EFFECT OF KETAMINE, A NMDA-RECEPTOR ANTAGONIST, ON SPONTANEOUS LOCOMOTOR ACTIVITY IN RATS

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The anesthetic ketamine interacts selectively with the N-methyl-D-aspartate (NMDA) receptor-channel complex, an excitatory amino acid receptor complex. We investigated the effect of different dosages of ketamine (3, 6, 12 mg/kg i.p.) on spontaneous locomotor activity and on intra-session habituation in an automated tunnel maze. Wistar rats (10/group) were tested first in a maze without any barriers and then in a 6-arm radial maze for three and five days, respectively (6-min session). Through the first period locomotor activity increased with the lowest and decreased with the highest dose of ketamine, whereas the intermediate dose remained ineffective. Complete tolerance developed through the second period, both for stimulation and depression. Intra-session habituation of maze locomotion was unaffected by the treatments. Contrary to the expectations, cognitive performance as estimated by measures of patrolling efficiency was not affected within this dose range.

PHY 325

EXCITATORY AMINO ACID (EAA) RECEPTORS IN THE LATERAL HABENULA (LHB) OF THE CAT. AN EXTRACELLULAR IONTOPHORESIS STUDY, IN SITU.

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The EAA agonists N-methyl-D-aspartate (NMDA), quisqualate (QUIS) and kainate (KA) and the EAA antagonists 2-amino-7-phosphono-heptanoate (AP-7) and kynurenate (KYAC) have been iontophoretically applied to single neurons in the lateral habenula of halothane anaesthetized cats and the effects measured by extracellular recording techniques. NMDA, QUIS and KA increased the spontaneous firing rate of the large majority (91%, 89%, 100%, respectively) of recorded LHb neurons. All the agonists elicited a regular firing pattern. Bursty firing pattern could be elicited by NMDA in non-LHb (i.e. thalamic or periaqueductal) neurons only. AP-7 a specific NMDA receptor antagonist inhibited the NMDA evoked excitation with much weaker effect on QUIS or KA excitation and no effect on spontaneous activity of LHb cells. KYAC a broad spectrum EAA antagonist however inhibited the effects of NMDA, QUIS and KA and reduced the spontaneous firing rate of LHb cells. These results indicate that a) most of the LHb cells possess EAA receptors of the NMDA and non-NMDA subtypes and b) spontaneous firing in LHb neurons might partly be mediated by non-NMDA EAA receptors.

PHY 326

TUMOR PROMOTERS ENHANCE THE DIFFERENTIATION OF ASTROCYTES IN A THREE-DIMENSIONAL CULTURE SYSTEM

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It has been shown previously (P. Honegger, J. Neurochem. 46, 1986, 1561-1566) that in reaggregating fetal brain cell cultures C-kinase-activating tumor promoters greatly enhance the maturation of astrocytes. In contrast, Murphy et al. (Devl. Brain Res. 31, 1987, 133-135), working with primary astrocyte monolayer cultures, reported that phorbol esters have a strong mitogenic effect. In a more detailed study with the C-kinase-activator mezerein, we were able to confirm our previous observation of a great enhancement of astrocyte differentiation and a lack of mitogenic activity in our 3-dimensional culture system. Furthermore, in such cultures pretreated with epidermal growth factor (EGF, 5 ng/ml), mezerein (50 nM) potentiated the synchronization of the mitotic activity as well as the subsequent acceleration of astrocyte differentiation both induced by EGF.

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PHY 327

HEAD-LEG-TRUNK MOVEMENT COORDINATION UNDERLYING EQUILIBRIATING REACTIONS IN MAN

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The coordination of leg, trunk and neck muscles was examined in normal adults. Postural sway was elicited in two ways: 1) Toe up rotation of the support surface about the ankle joints, stretching the triceps surae muscles and tipping the head backwards. 2) Rearwards translation of the support surface producing an equivalent triceps surae stretch but tipping the head forwards. The equilibriating reactions occurred with 120 to 140 ms onset latencies in all muscles without showing a proximal to distal activation order. This suggests that the control of head and body motion is coordinated on a feedforward basis to take into account differences in transmission times between afferent input and efferent muscle output. The largest responses always occurred in muscles which would stabilize sway. Since these muscles were either on the ventral or dorsal surface of the body for the two stimulus conditions and the same amplitude and direction of triceps stretch was imposed under both conditions, this implies that the vestibulo-genic signals underly the sway stabilizing responses.

PHY 328

EFFECTS OF PENTOBARBITAL AND KETAMINE ON THALAMIC AND CORTICAL AUDITORY NEURONS.

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Multiple spike trains were recorded in the auditory thalamus (78 units) and in the auditory cortex (61 units) of 14 nitrous oxide anaesthetized cats before and after the action of : i) Nembutal (7 mg/Kg); ii) Ketalar (12 mg/Kg); iii) both anaesthetics together. Each unit was caracterized by the statistical analysis of its spontaneous and acoustically driven activity. The anaesthetics affected differently the measured parameters. In the cortex the dominant effect of Nembutal was a decrease of the spontaneous firing rate and an increase of the signal/noise ratio. The Ketalar increased (37 %) and a decreased (87 %) these parameters respectively. In the thalamus the majority of the units (80 %) increased their tendency to fire in bursts after the Nembutal injection whereas the maximum instantaneous discharge frequency evoked by noise bursts tended to decrease (60 %). The effect of Ketalar was just the opposite by decreasing the former and increasing the latter.

PHY 329

AUTORADIOGRAPHIC LOCALIZATION OF OXYTOCIN AND VASOPRESSIN BINDING SITES IN THE HUMAN BRAIN. Loup F., Tribollet E., Dubois-Dauphin M., Pizzolato G. and Dreifuss J.J. Depts. Physiol., and Pathol., Univ. Med. Ctr., CH-1211 Geneva 4.

Using 3 nM tritiated oxytocin (OT) and 1.5 nM tritiated arginine vasopressin (AVP), specific binding sites have been visualized by in vitro light microscopic autoradiography in the brain and kidney of human subjects, 40-81 years old and without any history of neurological or psychiatric disease. The specificity of binding was assessed in competition experiments using synthetic structural analogues. High densities of [^{3}H]AVP binding sites were observed in the septal region, the parafascicular nucleus of the thalamus, the anterior lobe of the pituitary gland (but none in the posterior lobe), the choroid plexus and in both cortex and medulla of the kidney. Intense binding of [^{3}H]OT was found in the magnocellular basal nucleus, the substantia nigra pars compacta, the dorsal vagal complex, the hypoglossal nucleus, the spinal trigeminal nucleus and the substantia gelatinosa of the upper cervical spinal cord. Vasopressin and oxytocin, which are believed to act as neurotransmitters in the rodent nervous system, may thus play an important role in the human brain.

PHY 330

COLD-ACCLIMATION OF OBESE (fa/fa) ZUCKER RAT RESTORES ITS HYPERTHERMIC RESPONSE TO PREPONTINE MICROKNIFE LESION.

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In urethane-anesthetized lean Zucker rats, a microknife lesion in the prepontine region induced steady state hyperthermia of 3.5 °C, as a result of marked activation of brown adipose tissue (BAT). The temperature gradient between interscapular brown adipose tissue (IBAT) and colon, which was negative before the lesion, reversed a few min after the lesion and IBAT fractional blood flow increased 12-fold. The same responses could be mimicked by noradrenaline infusion. By contrast, in the obese animal, no hyperthermia was measured after either the prepontine lesion or noradrenaline infusion despite a 4-fold increase in BAT blood flow. The hyperthermic responses to noradrenaline or to the lesion were both restored in three-week cold-acclimated obese animals. It is concluded that the lack of response of the obese animal kept at room temperature is due to an involution of BAT resulting from a central tonic inhibition of nonshivering thermogenesis.

PHY 331

DOES ADRENALECTOMY AFFECT THE HYPERTHERMIC RESPONSE TO PREPONTINE MICROKNIFE LESION IN THE RAT?

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In urethane-anesthetized rats, prepontine knife lesion induced a long lasting hyperthermia mostly due to an increase in sympathetic stimulation of brown adipose tissue (BAT) thermogenesis that had been triggered by the suppression of tonic inhibitory control of its heat production. Since both circulating adrenaline and noradrenaline were markedly increased by urethane and further increased by the lesion, the effect of adrenalectomy was studied. Compared to control rats, the initial (30 min) rate of rise of colonic temperature following the lesion was the same, whereas the duration was shorter and the maximal temperature increase lower in adrenalectomized rats. By contrast, the interscapular BAT temperature was much higher and sustained and BAT fractional cardiac output was larger than in controls. Increased temperature gradient together with increased perfusion indicate a greater heat production by the tissue. It is concluded that adrenalectomy decreased the hyperthermic response despite an increased thermogenic capacity of BAT. Both glycemia and insulinemia were found to be lower and this might contribute to the decrease in extra BAT heat production in adrenalectomized rats.

PHY 332

VASOPRESSIN EXCITES NEURONES IN THE DORSAL COCHLEAR NUCLEUS OF THE GUINEA-PIG BRAIN. Charpak, S., Dubois-Dauphin, M., Tribollet, E. and Dreifuss, J.J. Dept. of Physiology, University Medical Center, CH-1211 Geneva 4.

Immunoreactivity to the vasopressin-associated glycopeptide has been detected in neurones located in the guinea-pig brainstem. Immunoreactive fibers are present in the dorsal cochlear nucleus in sexually immature animals of both sexes; in the adult immunoreactivity was only detected in the female (Dubois-Dauphin et al., Experientia, 43, 712, 1987). To assess whether neurones in the cochlear nucleus are sensitive to vasopressin, coronal slices were prepared from young or old guinea-pigs of either sex. Intracellular recordings were obtained from dorsal cochlear neurones and the slices perifused with arginine vasopressin (AVP), oxytocin (OT) and with receptor-specific agonists. Amongst 33 neurones exposed to 10-1000 nM AVP, 27 increased their rate of firing; the remainder were unaffected. OT excited 17/21 neurones. No obvious differences in chemosensitivity were apparent depending upon the age or the sex of the animals. Preliminary results suggest that dorsal cochlear neurones possess V_1 vasopressin receptors and/or oxytocin receptors.

PHY 333

AUTORADIOGRAPHIC LOCALIZATION OF OXYTOCIN BINDING SITES IN THE DEVELOPING RAT BRAIN. Tribollet, E., Dubois-Dauphin, M., Loup, F., Charpak, S. and Dreifuss, J.J. Dept. of Physiology, University Medical Center, CH-1211 Geneva 4.

High affinity binding sites for oxytocin have been detected in the embryonic and neonatal rat brain using a recently synthetized and radioiodinated oxytocin analogue. The specificity of binding was assessed in displacement studies with unlabelled oxytocin. Binding was first observed at day E17, where it was restricted in the dorsal motor nucleus of the vagus nerve. From then, additional structures are progressively detected. Ten days after birth, the binding site distribution is still not the same as in the adult. The most important differences are 1) intense labelling in regions poor or devoid of binding sites in the adult brain, namely the striatum, the retrosplenial agranular cortex, the dorsal hippocampus, the lateral reticular nucleus, the spinal trigeminal nucleus; 2) diffuse specific labelling throughout the hypothalamus and amygdala; and 3) the low density of sites in the hypothalamic ventromedial nucleus. Preliminary electrophysiological studies suggest that the oxytocin sites present in neonatal rats might represent functional receptors.

PHY 334

IN A COMPARTMENTED NERVOUS SYSTEM GLIA TRANSFORM GLUCOSE AND SUPPLY THE NEURONS WITH METABOLIC SUBSTRATE

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About one hundred years has elapsed since Golgi proposed that glial cells supply neurons with nutrients, yet no direct evidence supports this important hypothesis. This evidence is provided now from work on the honeybee drone retina, a nervous system in which glial cells and photoreceptor cells (sensory neurons) constitute two distinct metabolic compartments. Retinal slices incubated with ^3H 2-deoxyglucose phosphorylated this glucose analogue to ^3H 2-deoxyglucose-6P, but this conversion is made only in the glial cells. Hence, glycolysis occurs only in glial cells. In contrast photoreceptor cells consume O_2 and this consumption is sustained by a glycolytic intermediate supplied by the hydrolysis of glycogen contained in larger amounts in the glial cells. When the photoreceptor cells are photostimulated, their O_2 consumption quadruples and glucose metabolism in the glia is enhanced to meet the substrate requirements of the activated photoreceptors. This is a clear case of metabolic interaction between neurons and glial cells.

PHY 335

EFFECT OF THE ANTHELMINTIC AVERMECTIN B_{1a} ON THE VERTEBRATE GABA-RECEPTOR CHANNEL

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Neuronal GABA-receptor channels were expressed in Xenopus oocytes after injection with chick brain total mRNA. In this system GABA induced chloride currents can easily be measured under voltage clamp conditions. The quantitative effect of the anthelmintic Avermectin B1a on this channel was studied. GABA induced chloride currents were enhanced by the drug in a dose-dependent manner, with a half-maximal effect at 0.1μM. Avermectin B_{1a} did not affect the reversal potential of the current or alter the membrane permeability in the absence of GABA. The major effects of the drug were a shift in the K_a for GABA from 21 to 2μM, a decrease in the Hill-coefficient for GABA from 1.7 to 1.1, and a strong inhibition of GABA induced desensitization of the current. When the oocytes were exposed for several tens of minutes to Avermectin, the drug also caused a slow rundown of the maximal current amplitude. Furthermore, it was shown that Avermectin did not act at any of the known allosteric modulatory sites for benzodiazepines, picrotoxin or barbiturates.

PHY 336

PRIMARY CULTURE OF NEURONES FROM THE CENTRAL NERVOUS SYSTEM OF FRESHWATER CRAYFISH

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We have developed a primary cell culture system for neurones from the central nervous system of two species of freshwater crayfish, Procambarus clarkii and Pacifastacus leniusculus. Neurones were isolated by suction after dissection of abdominal ganglia and subsequent treatment with collagenase/dispase. Isolated cells were plated under sterile conditions on poly-L-lysine-coated glass coverslips which were incubated in plastic multiwells containing supplemented L-15 medium with 5% fetal calf serum and 50 µg/ml gentamycine. Under these conditions neurones survived for at least 10 days and many of them grew neuritic processes of different length, diameter, and degree of branching. Growth started as early as about 10 hours after plating. Growing as well as non-growing cells had characteristic electrophysiological properties like high resting membrane potential (-50 to -70 mV), high input resistance (about 40 MΩ), and most of them were electrically excitable.

PHY 337

HYPOTHALAMIC SEROTONIN TURNOVER IN ROMAN HIGH- AND LOW-AVOIDANCE (RHA/VERH AND RLA/VERH) RATS

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RHA/Verh rats are selected and bred for the rapid acquisition of active, 2-way avoidance, and RLA/-Verh rats are selected and bred for the non-acquisition of that response. Basal turnover rates of 5-HT were compared in 3 brain regions involved in those behaviors. Using adult males, and the methods of a previous study which measured the disappearance of the metabolite 5-HIAA after MAO inhibition by pargylene, at 4 time points (Driscoll et al. Eur. J. Pharmacol. 68, 373, 1980), it was found that RHA/Verh rats had a hypothalamic 5-HT turnover rate almost double that of RLA/Verh rats. No differences were seen in the striata or hippocampus. Whereas RHA/Verh rats have a higher, basal hypothalamic turnover, however, they actually show an attenuated 5-HT metabolism and/or synthesis after acute footshock stress, a procedure which provokes an increase of 5-HT metabolism in RLA/Verh rats (Driscoll et al. Life Sci. 33, 1719, 1983).

PHY 338

ELECTROPHYSIOLOGICAL STUDY OF FACIAL MOTONEURONS IN VITRO AND IN VIVO.

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The properties of cranial motoneurons innervating somatic musculature in the ***adult mammal*** have not so far been studied in vitro. Such an approach is nevertheless needed when one considers to apply the appropriate pharmacological tools. The isolated and perfused whole brain of guinea pig allows to study the intrinsic membrane properties of neurons that have been antidromically identified and activated synaptically.The aims of the following study were thus twofold: **First** to evaluate the survival of identified brainstem motoneurons in that new preparation. Facial motoneurons were therefore considered as good candidates. Few data being available on that specie we found necessary to first compare the properties of these cells in vivo and in vitro. **Second** we investigated in particular whether these cells demonstrate calcium conductances .Our results show that the properties of facial motoneurons in the isolated brain are similar to those obtained from motoneurons recorded in acute animals of the same specie. The main result of the pharmacological study of facial motoneurons is the presence of high threshold calcium spikes of a presumed dendritic origin. This could be significant for understanding the way cranial motoneurons integrate incoming signals.

PHY 339

ELECTROPHYSIOLOGICAL STUDY OF RETICULAR NEURONS IN AN ISOLATED WHOLE BRAIN OF GUINEA PIG AND IN BRAINSTEM SLICES

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Ponto-medullary reticular neurons belong to networks subserving heterogenous functions. Little attempts have yet been made to study their membrane properties in vitro. Our goal was to find out whether this functional heterogeneity is reflected in the intrinsic properties of these neurons. We therefore studied a circumscribed area which is part of Nucleus Gigantocellularis (NGC). This area is known to contain a high density of reticulospinal neurons. We aimed first at classifying the different cell types in an isolated whole brain preparation. Second we undertook a pharmacological study in brainstem slices. Using these two techniques we have identified so far 3 neuronal cell types in the NGC. They differed by their action potential width and rise time, their membrane input resistance and responses to pharmacological agents.1) Type 1 had a medium broad action potential with a slower risetime followed by a single afterhyperpolariztion. It had a higher input resistance and displayed high threshold calcium dependant spikes and an A type of rectification (in a majority of cells). 2)Type 2 had a very thin and fast action potential followed by an early fast and a delayed slow after-hyperpolarization. It had a lower input resistance and also displayed high threshold calcium dependant spikes and plateau potentials.3)Type 3 had a very broad action potential, due to a strong calcium dependant component, and fired regularly in an oscillatory manner.

PHY 340

FUNCTIONAL ORGANIZATION OF THE VENTRAL DIVISION OF THE MEDIAL GENICULATE BODY (MGB) OF THE CAT AND ARRANGEMENT OF ITS RECIPROCAL CONNECTIONS WITH THE AUDITORY CORTEX.

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The location of 2152 single units in the ventral division of the MGB was correlated with their responses to sounds. Going from rostral to caudal, perpendicular to the best frequency gradient, the response latencies became longer and more variable, the proportion of inhibitory responses increased, the responsiveness to broad band stimuli decreased, the tonotopic arrangement was less strict and units became more broadly tuned. Following injections of the tracer WGA-HRP in the anterior (AAF) and primary (AI) auditory cortical fields, labeled cells and corticofugal terminals overlaped in the anterior half of the ventral division of the MGB whereas labeling was seen in its posterior half with injection in the posterior (PAF) cortical field. Taken together, these data suggest that the anterior half of the ventral division of the MGB transfers to AI and AAF a well preserved auditory information from the periphery whereas its posterior half processes a more complex information in cooperation with PAF.

PHY 341

INTRACELLULAR CALCIUM ENHANCES SODIUM CURRENT IN NEUROBLASTOMA CELLS

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Sodium currents in mouse neuroblastoma cells of the clone N1E-115 were investigated using the whole-cell configuration of the patch-clamp method. Cells were cultured in DMEM containing 10% FCS in a 10% CO_2-atmosphere and subjected to experiments 1 day after plating on plastic petri-dishes. Experiments were carried out at a fixed temperature of 20°C. Whole cell recording was established with glass electrodes with resistances of about 1 Mohm and series resistance was compensated electronically. A holding potential of -70 mV was used to maintain the majority of channels in the activatable state. Peak Na currents were recorded at regular intervals by depolarizing the cell membrane to 0 mV for 20 ms. During a period of about 40 minutes Na currents showed a constant increase due to their recovery from slow inactivation. A variety of Ca buffered solutions with a free Ca concentration within the physiological range was used and compared to a virtually Ca free pipette solution. Membrane currents were significantly higher in the presence of physiological Ca concentrations inside the cells compared to cells containing practically no free Ca.

PHY 342

UPTAKE OF ADENOSINE AND BINDING OF ADENOSINE ANALOGUES IN CULTURES OF RAT CEREBELLUM AND SPINAL CORD: AN AUTORADIOGRAPHIC STUDY.

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Autoradiographic studies have shown that ^{3}H-adenosine - a putative neurotransmitter or neuromodulator - was taken up by many neurones and astrocytes in cerebellar and spinal cord cultures. The uptake of adenosine was inhibited in the absence of sodium or at 0°C, suggesting an active transport mechanism.
Binding sites for the A_1-receptor agonist ^{3}H-R-N^{6}-phenylisopropyladenosine and for the mixed A_1/A_2-agonist ^{3}H-N(ethyl) carboxamidoadenosine were observed on a great number of spinal and cerebellar neurones. Binding of both radio-ligands was also found on astrocytes, suggesting that glial cells have receptors for adenosine. This observation is further supported by electrophysiological studies demonstrating that adenosine and its analogues cause hyperpolarizations of astrocytes which are blocked by the adenosine antagonist theophylline (Hösli et al. Neurosci. Lett. 79: 108-112, 1987).

PHY 343

DENDRITIC TREE OF MOTONEURONS GROWN IN VITRO: BRANCHING STRUCTURE, EXTENT AND COMPLEXITY IN RELATION TO SOMA SIZE

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Developing neurons apparently adapt their dendritic growth to the various influences of neighboring cells and to the inputs they receive. Motoneurons (MNs) were grown in an organotypic culture of rat spinal cord from embryonic day 14 along with coexplanted skeletal muscle. After retrograde labelling with HRP, the dendritic trees of 3 MNs were examined on day 15 and 3 MNs on day 20 *in vitro*. Each stem dendrite branched dichotomously 2-6 times to form an apparently random binary tree. Mean dendritic lengths in the 6 MNs ranged from 182 to 323 µm and total dendritic lengths from 1109 to 3146 µm. Both were positively correlated with soma size. Two other measures of dendritic complexity also increased with the size of the soma: (1) the total number of terminal segments and (2) the ratios between the total numbers of terminal segments and the numbers of stem dendrites. A vertex analysis suggested that, like the Purkinje dendrites studied by Berry and Flynn (Proc. R. Soc., Lond. B, 221, 1984), these dendritic trees probably developed by terminal growth that was at least partially random. The less complex dendritic trees of these MNs may be due to reduction of synaptic input in vitro. Supported by SNF 3.265.0.85.

PHY 344

ROLE OF SINGLE-FIBER EXCITATORY POSTSYNAPTIC POTENTIALS IN PARTITIONING AND FINE TUNING OF STRETCH REFLEXES

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Spike-triggered averages of excitatory postsynaptic potentials elicited by impulses in single stretch-afferent fibers from the medial gastrocnemius muscle were recorded intracellularly from homonymous and heteronymous motoneurons (MNs). The amplitudes of these single-fiber e.p.s.p.s were related directly and exponentially to the conduction velocities of the Ia or spindle group II afferents. The closer a MN was to the spinal entry point of an afferent fiber, the larger was the mean e.p.s.p. evoked in it. Impulses in the same afferent fiber elicited larger e.p.s.p.s in small than large MNs that were located at the same cranio-caudal levels. Other factors being equal, e.p.s.p.s in homonymous and heteronymous MNs were of equal amplitude. Thus, rather than species differences, morphological and topographical factors, such as the sizes of the afferent fibers and MNs and the distances entering fibers must travel to reach MNs, apparently account for these variations in e.p.s.p. amplitude. Accordingly, when local perturbations in muscle occur, the larger e.p.s.p.s in MNs near the entry points of the discharging afferents cause them to fire back to the perturbed region. Preservation of orderly recruitment in these "partitioned" reflexes guarantees finely tuned stretch reflexes regardless of the location and magnitude of intramuscular disturbances. Supported by SNF 3.265.0.85

PHY 345

EXCITATORY ACTION OF VASOPRESSIN IN THE LATERAL SEPTUM.

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Extracellular recordings were obtained using slices from the rat brain in vitro. Of 177 neurones tested in the presence of vasopressin, 78 responded by a reversible increase in firing rate, 12 were inhibited and the remaining were not affected. The lowest peptide concentration effective ranged between 1 and 100 nM, and the magnitude of the excitatory effect was concentration- dependent. At high vasopressin concentrations, the peptide-induced excitation was often followed by a transient pause in firing; this was probably due to action potential inactivation, brought about by the vasopressin-induced neuronal membrane depolarization. The excitatory effect of vasopressin was postsynaptic, since it was not abolished following synaptic blockade in a low calcium-high magnesium perifusion solution. Pharmacological data showed that most of the septal vasopressin-sensitive neurones are endowed with vasopressin receptors of the V_1 (or vasopressor) type, whereas a minority of them bear oxytocin receptors. Our results favour the notion that vasopressin may play a neurotransmitter role in the lateral septum.

PHY 346

ON AND OFF LAMINATION IN THE RETINO-GENICULO-STRIATE PATHWAY OF TUPAIA: A PARAMETRIC STUDY

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The goal of the present study was to investigate the ON and OFF characteristics of **single cells** in the visual pathway of tree shrews (Tupaia belangeri). We recorded from neurons in the dorsal lateral geniculate nucleus (dLGN) and the striate cortex (A17).
Our **single unit recordings** from the dLGN (n=29) and the A17 (n=47) confirm the functional subdivision. In the dLGN: laminae 1 and 2 neurons show an ON response characteristic, whereas laminae 3, 4 and 5 neurons show an OFF response characteristic. Lamina 6 neurons respond either to light on, off or both. Laminae 1 and 5 cells are driven ipsilaterally, the others contralaterally. In A17: layer IVa is a predominantly ON response region (87%), and layer IVb a predominantly OFF response region (87%). The remaining neurons exhibit ON-OFF characteristics. Supragranular layer III contains ON, OFF and ON-OFF cells, while in infragranular layer V, ON-OFF cells have been found only.
These results demonstrate separate ON and OFF channels in the mammalian visual system even on the single unit level.

PHY 347

DOPAMINERGIC CONTROL OF GABA TURNOVER IN MICE

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The influence of dopamine agonists and antagonists on GABA turnover was estimated in four regions of mouse brain (cortex, cerebellum, hippocampus and c.striatum) by measuring the accumulation of GABA after GABA-T inhibition with gabaculine. Apomorphine, a mixed D1/D2- agonist, reduced GABA synthesis by about 50%. Similarly, PPHT (2-(N-Phenyl- ethyl-N-propyl) amino-5-hydroxytetralin), a selective D2-agonist, dose-dependently reduced GABA turnover and S-sulpiride, a D2 antagonist, blocked the effect of apomorphine and PPHT.
By contrast, the preferential D1-agonist, SKF 38393 had no effect on GABA turnover, and the selective D1-antagonist, SCH 23390, was without effect on the apomorphine-induced reduction in GABA turnover or slightly increased it. It is concluded that primarily D2 receptors mediate the effect of DA on GABAergic transmission. This interaction appears to be DA receptor specific as the activity of apomorphine was not influenced by idazoxan and propranolol.

PHY 348

PLASMA GLUCOSE CHANGES INDUCED BY NOREPINEPHRINE INJECTION IN THE PARAVENTRICULAR NUCLEUS (PVN) IN LEAN AND OBESE (FA/FA) RATS.

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Laboratoires de Recherches Métaboliques, Geneva, Switzerland

The effect of norepinephrine (NE) injection into the PVN of the hypothalamus on plasma glucose and insulin levels was studied. The rats were equipped with chronic right jugular catheters for blood sampling. Two min after NA injection (40 mmoles), plasma glucose rose, reaching a peek at 10 min in the lean rats, without any change in insulinemia. In obese rats, the glycemia started to increase at 4,5 min post-stimulus and the levels were reduced but not abolished. To conclude, these findings show that the obese rats are less sensitive to a sympathetic stimulation than the lean rats.

PHY 349

INFLUENCE OF Cs OR TMA ON SODIUM GATING AND IONIC CURRENTS IN THE SQUID GIANT AXON:

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When recording Na gating currents with 350 mM Cs as the internal substitute for K, a slow ionic current, presumably Cs leaking through K channels,appears at pulse potentials above 20 mV. This contamination is suppressed when using 350 mM Tetramethylammonium (TMA) as the internal cation. Consequently a comparative study of the effect of either Cs or TMA perfusion on Na gating and ionic currents was carried out. The Na gating currents were found to be the same with respect to size and time course in either perfusate. The series resistance is about 50% larger in TMA and can lead to slight rounding of the gating current rising phase when not properly compensated. Na ionic currents were measured with 103 mM Na externally and 20 mM Na, 330 mM Cs or TMA internally. TMA was found to decrease the peak Na current and to increase the non-inactivating Na current confirming other studies (Oxford &Yeh,J.Gen Physiol.,1985,85,583). Instantaneous current steps during the non-inactivating phase show a voltage dependent blocking of Na channels above the Na reversal potential in TMA which is practically absent in Cs.

PHY 350

SINGLE CHANNEL CURRENT SIMULATION AND RECORDING USING A PHOTODIODE AS CURRENT GENERATOR

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A device which can generate rectangular currents in the pico ampere range is described. This current generator consists of a photodiode connected to the headstage of a single channel recording amplifier. The activation of the photodiode is mediated by a light emitting diode which can be controlled by a computer or other suitable current source. One application of this current generator is the tuning of patch clamp amplifiers: rectangular currents from the photodiode allow the frequency response of the amplifier to be correctly adjusted. Alternatively the light emitting diode can transmit signals corresponding to simulated single channel behaviour. Since the kinetic parameters of the simulation are known, the user can test the data acquisition and analysis system under conditions similar to those prevailing during recording from a biological membrane. The hardware and software aspects of this system will be presented with examples of single and multichannel simulations.

PHY 351

FAST COMPONENTS OF MEMBRANE ASYMMETRY CURRENTS IN THE SQUID GIANT AXON.

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Recording of gating currents associated with Na channels in response to a voltage step from freshly mounted squid axons using a wideband low noise voltage clamp reveals a fast asymmetrical charge movement superimposed upon the normally observed gating current. Studies using a linear model membrane showed that the charge movement is not due to non-linearities in the voltage clamp. The finding is consistent with the hypothesis that the axon is inhomogeneously clamped with part of the membrane being overcompensated resulting in a transient overshoot of the desired membrane potential not observable in the capacitive transient itself. Candidate lumped equivalent electrical models of the membrane which could account for the observations will be discussed. These findings indicate that care must be taken in interpreting gating charge movement during the first 30 µs of an applied voltage step.

PHY 352

THE AFTER-POTENTIALS IN RABBIT NONMYELINATED NERVE FIBRES

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The action potentials in vagus C fibers were recorded by the sucrose-gap method. We have observed that the spike is followed by three afterpotentials: a fast hyperpolarisation, (fast.a.h.p),lasting 50-100ms, an after depolarisation, (100-200 ms) and a slow hyperpolarisation, (slow.a.h.p.), lasting 500ms - 1s. Both the fast and the slow a.h.p depend on the electrochemical gradient of K^+ and on the extracellular concentration of Ca^{2+}. They are abolished by TEA (50 mM), and by the calcium entry blocker Cd^{2+} (1mM), and enhanced in presence of 4-AP (5-100 µM). The slow but not the fast a.h.p. is blocked by noradrenaline (10 µM), indicating that the slow and the fast a.h.p. are mediated by two different Ca^{2+}-activated K^+ channels. The repolarisation of both a.h.p is strongly accelerated in nerves loaded with the intracellular Ca^{2+} buffer quin2, indicating that the rate limiting step in the generation of the a.h.p are the changes in the concentration of intracellular Ca^{2+}. Application of Mg^{2+} (10 mM) produces a rapid inhibition of the fast a.h.p. and a transient enhancement of the slow a.h.p., but a prolonged (30 min) application of this ion results in a decrease of the slow a.h.p. also.

PHY 353

IN VITRO RELEASE OF N-ACETYLASPARTYLGLUTAMATE UPON WHOLE TISSUE DEPOLARIZATION OF BRAIN SLICES

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Electrophysiological, immunohistochemical and pharmacological evidence favours a neurotransmitter role of N-acetylaspartylglutamate (NAAG). Demonstration of NAAG release upon depolarization is still missing. We have developed an analytical method for quantifying NAAG in in vitro perfusates of brain slices, consisting of an ion-exchange prepurification, followed by derivatization and GCMS with chemical ionization and single ion monitoring. Deuterated NAAG was used as an internal standard. Detection limits of 1 pmol and lower can be achieved. In vitro release of NAAG from rat neocortex, pyriform cortex and hippocampus upon depolarization with 50 mM K^+ could be demonstrated and was shown to be Ca^{++} dependent. Typical amounts of NAAG from cortex were 2 pmol/ mg protein and min. in stimulated fractions compared to 0.6 pmol/ mg min. under resting conditions. These results support the hypothesis that NAAG is a transmitter candidate in cortical structures.

PHY 354

EFFECT OF CLIMBING FIBERS DEGENERATION ON IN VITRO RELEASE OF ENDOGENOUS GLUTAMATE, ASPARTATE, HOMOCYSTEATE AND ADENOSINE IN RAT CEREBELLUM

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The transmitters and modulators of the olivo-cerebellar climbing fiber (cf) pathway are still a matter of debate. The K^+-induced, Ca^{++}-dependant in vitro release of neuroactive substances has been investigated in control and cf deprived cerebella. Degeneration of the inferior olive was induced by 3-acetylpyridine (3-AP). Glutamate (Glu), aspartate (Asp), homocysteate (HCA) and adenosine (Ado) were determined by reversed phase HPLC. HCA release was abolished after 3-AP. In hemisphere but not in vermis of 3-AP treated rats, a decrease in stimulated release of Asp by 49% and of Glu by 14% was observed. Glu basal release was increased by 60%. A cerebellar release of Ado was established and decreased by 60% in 3-AP treated animals. It is suggested that cf are using HCA, Asp and/or Glu as transmitters. Ado, as neuromodulator, might presynaptically reduce Glu release from parallel fibers.

PHY 355

DEVELOPMENTAL CHANGES OF NMDA RECEPTORS IN CHICKEN CERBELLUM.

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In adult rat, pigeon and chicken cerebellum, no functional N-methyl-D-aspartate (NMDA) receptors can be found in [$^{22}Na^+$] efflux experiments. Around hatching, however, a considerable NMDA stimulated [$^{22}Na^+$] efflux was present in chick cerebellar slices. In frozen/thawed chick cerebellar homogenates NMDA displaceable L-[^{3}H]-glutamate binding was first detectable after incubation day 14, reached its maximum at hatching and decreased to about 30% of this level 3 weeks later. The time-course of this decrease was similar to that found in [$^{22}Na^+$] efflux experiments. The time-course of the increase, on the other hand, was the same as that observed for specific L-[^{3}H]-glutamate binding which also reached its maximum at hatching but in contrast did not decrease thereafter.
NMDA receptor decrease after hatching and the maintenance of maximal levels of specific L-[^{3}H]-glutamate binding suggest that NMDA receptors would be replaced postnatally by other types of binding sites for excitatory amino acids in chicken cerebellum.

Biochemistry (BIO)

BIO 356

ADDITION OF GLYCOPHOSPHOLIPID ANCHORS TO A MAJOR 120 kDa MEMBRANE PROTEIN IN SACCHAROMYCES CEREVISIAE.

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A number of externally exposed plasmamembrane glycoproteins of mammalian and protozoan origin has been shown to be anchored in the membrane through a novel and structurally complex glycophospholipid which contains phosphatidylinositol, mannose, glycosamine and ethanolamin. We used biosynthetic labeling procedures to demonstrate that a major 120 kDa membrane glycoprotein of S. cerevisiae contains a similar anchor. An experiment with the secretion mutant sec 18 which does not transport glycoproteins from the RER to the Golgi at 37°C indicates that the protein is made as a larger precursor and receives its lipid anchor in the RER.

BIO 357

CROSSREACTION OF ANTI-PORCINE PANCREAS PLA_2 ANTIBODIES WITH BOVINE ADRENAL MEDULLARY PROTEINS

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Antisera against SDS-denatured and native, iodoacetamide-inactivated porcine pancreas phospholipase A_2 (PLA_2) were produced in rabbits. Antiserum against the SDS-denatured PLA_2 recognizes PLA_2 from porcine pancreas as well as from the Elapid snake Naja naja. Antiserum against the native PLA_2 in addition even recognizes the PLA_2 from the Viperid snake Crotalus durissus. The latter antiserum was found to crossreact with two proteins of the bovine adrenal medulla on Western blots. The two proteins, of MW 50 kD and 70 kD, are detected in the crude plasma membrane fraction but are absent from chromaffin granules and the soluble fraction. They are not present in the crude plasma membrane fraction of bovine liver.
The antiserum may be helpful in the detection of a chromaffin cell PLA_2-activity which is supposed to be functionally involved in the exocytotic release of hormone from these cells.

BIO 358

A novel heterodimeric structure of 135 and 145 Kd possibly involved in T cell activation.

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The T cells protein (Tp135-145) was identified by a monoclonal antibody, Mx24, on the surface of both T3/TCR$^+$ and T3/TCR$^-$ human T cell lines. Mab Mx24 precipitated two major bands of 135 and 145 Kd both under reduced and nonreduced conditions from lysates of I-125-surface labeled cells. Modulation of Tp135-145 had no effect on the expression of T3 and of the TCR. Conversely, the expression of Tp135-145 was not affected by modulation of the T3/TCR molecular complex by either anti-T3 or anti-TCR antibody.
Functional studies showed that Mab Mx24 induced high levels of IL-2 production in Jurkat cells. In addition, anti-Tp135-145 mAb was found to be mitogenic for a subpopulation of peripheral blood T cells. FACS analysis showed that only 28-76% of E-rosette-positive cells were stained with Mab Mx24. In addition, multicolor flow cytometry analysis showed that the Tp135-145$^+$ cells belong to both the T4 and T8 subsets.

BIO 359

CD3-ASSOCIATED α/β AND γ/δ HETERODIMERIC RECEPTORS ARE EXPRESSED BY DISTINCT POPULATIONS OF CD4$^-$ CD8$^-$ THYMOCYTES

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The relationship of T cell lineages bearing α/β or γ/δ T cell receptors (TCR) is as yet unclear. Adult CD4$^-$ CD8$^-$ (DN) thymocytes contain precursors of mature CD4$^+$ and CD8$^+$ T cells and we now show that these DN thymocytes contain phenotypically distinct populations expressing either α/β or γ/δ TCRs. The α/β TCR$^+$ subset expresses only minimal levels of TCR δ mRNA, in agreement with a recently proposed deletional mechanism allowing for mutually exclusive expression of TCR α and δ genes. Both populations, however, unlike mature α/β TCR$^+$ T cells, have similar high levels of TCR γ mRNA. α/β TCR$^+$ DN cells may therefore represent an early stage after divergence of this lineage from precursors that would have failed to rearrange productively either the TCR γ or δ genes.

BIO 360

Opsonization by anti-band 3 antibodies: formation of C3b-IgG complexes on oxidatively stressed human red blood cells

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We have reported that naturally occurring anti-band 3 antibodies elicited C3b deposition and phagocytosis of oxidatively stressed, but not of control red cells (PNAS 84(1987) Nov.). Bound C3b exceeded bound antibody by two orders of magnitude. A large portion of nascent C3b was covalently bound to cell associated IgG rather than integral membrane components. The number of C3b-IgG complexes increased by increasing the concentration of anti-band 3 in serum. Anti-spectrin did not enhance C3b-IgG complex formation when antibody supplemented serum was preadsorbed on control red cells. The data suggest a selective binding of anti-band 3 to oligomerized band 3 proteins on oxidatively stressed red cells and activation of alternative pathway C3b deposition by bound anti-band 3. An alternative explanation, that anti-band 3 acted like an immune complex and activated C3b deposition to fluid phase IgG which subsequently bound as C3b-IgG to red cell CR1, is unlikely. Elevated concentrations of anti-band 3 also stimulated phagocytosis of oxidatively stressed red cells that were pretreated with anti-CR1 (Blood Cells, in press).

BIO 361

EXPRESSION OF THE TNF LOCUS IN MURINE LYMPHOCYTES

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The mouse TNF locus contains the genes for TNF-beta (lymphotoxin) and TNF-alpha (tumor necrosis factor) in a head-to-tail tandem arrangement, with only 1100 nucleotides separating the 3' end of the fourth TNF-beta exon from the 5' end of the first TNF-alpha exon. Nevertheless, the two genes are expressed with distinct tissue specificities, and are activated by different stimuli. We are investigating the molecular basis for this differential expression. Our observations so far can be summarized as follows : (i) In in vitro transcription reactions, the TNF-alpha promoter is stronger than the TNF-beta promoter; (ii) Nuclear run-on experiments also show a higher transcriptional activity in the TNF-alpha region than in the TNF-beta region, even in cells that contain more TNF-beta than TNF-alpha cytoplasmic RNA. We are currently testing the effects of the TNF-alpha and -beta promoter and polyadenylation regions on the expression of a linked bacterial CAT gene. Our results are consistent with the hypothesis that the tissue specificity of expression of the two components of the TNF locus is regulated at a post-transcriptional step.

BIO 362

MONOCLONAL ANTI-BAND 3 ANTIBODIES RECOGNIZING THE EXTERNAL SURFACE OF THE ANION EXCHANGE PROTEIN.

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Anti-band 3 monoclonal antibodies (mAb) have been produced by fusion of spleen cells from BALB/c mice ,immunized with human erythrocytes, and X63 Ag8.653 myeloma cells. Hybridoma cells were cloned and supernatants screened for anti-surface antigens by solid-phase ELISA techniques. Two out of 86 hybridoma cell lines were found to produce anti-band 3 mAb. Binding specificities have been tested by competitive ELISA and immunoblotting techniques utilizing immobilized erythrocytes and/or affinity purified band 3 protein as antigen. Anti-band 3 mAb have been characterized as IgG_{2b} and IgM respectively. With respect to domain specificity it was found that IgG mAb recognize an external surface epitope located within the 17 kD transmembrane domain, a subfragment of the N-terminal 55 kD chymotryptic segment of band 3.

BIO 363

IDENTIFICATION OF THE MSH RECEPTOR ON B16 MELANOMA CELLS BY PHOTOAFFINITY LABELLING

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α-MSH induces melanogenesis in murine melanoma cells by interaction with a specific plasma membrane receptor. We have synthesized a highly potent α-MSH photoaffinity label by specific attachment of the 2-nitro-4-azidophenylsulfenyl group to the tryptophan-9 residue of [norleucine-4, D-phenylalanine-7]-α-MSH. Iodination of this molecule yields a radioactive photolabel with a 2- to 4-fold higher bioactivity than that of α-MSH. The binding assay with intact B16 mouse melanoma cells reveals ~19,000 MSH receptors/cell and a K_D for the photolabel of ~0.5 nM. Peptide-receptor binding to intact B16 cells or plasma membranes followed by UV-irradiation, SDS-PAGE and autoradiography indicate that the photolabel inserts specifically into a single 45 kDa band. Additional experiments show that that the 45 kDa band is a glycoprotein and represents the MSH receptor or a subunit thereof.

BIO 364

GENE TRANSFER, MOLECULAR CLONING AND EXPRESSION OF THE HUMAN 4F2 ANTIGEN HEAVY CHAIN

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The human cell surface antigen 4F2 is a glycoprotein present in all cultured cell lines tested so far. Its expression in B or T lymphocytes is induced early after growth stimulation. The antigen is composed of a glycosylated heavy subunit of 85kDa linked by a disulfide bridge to a nonglycosylated 40kDa light chain. Its function has been suggested to correspond to the Ca^{++}/Na^{+} exchanger. We have isolated and sequenced a full-length cDNA coding for the human 4F2 heavy chain. Our results indicate that it is a transmembrane glycoprotein of 529 residues with a cytoplasmic NH_2 terminus and four N-linked glycosylation sites. The 4F2 heavy chain shows no homology to known protein sequences. We expressed the cDNA in mouse cells under the control of the cellular 4F2 promoter which we identified as a GC-rich region containing the GGCGGG motif of SP1 factor binding sites. Immunoprecipitations in transfected mouse L cells showed that the human 4F2 heavy chain forms a heterodimer with the mouse light chain. We are analysing its expression throughout the cell cycle and during T cell activation.

BIO 365

METHODS, USEFUL DETERGENTS AND THEORETICAL ASPECTS OF MEMBRANE PROTEIN CRYSTALLISATION

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The OmpF K-12 porin, an integral membrane protein from the outer membrane of E. coli that is involved in facilitated diffusion of small solutes, has been purified and crystallized in a similar way to E. coli B^m porin (M. Garavito), diffracting beyond 3.5 Å.
This protein is very resistant to denaturation, allowing us to test a wide variety of conditions. Over 20 detergents have been tested for their potential use in crystallization of membrane protein. Among the successful ones are long chains, zwitterionic and low cmc detergents. The most interesting ones are phospholipid analogs which provide for the first time a quasi-natural environment for the protein in the crystal. Further studies on the phase diagrams define conditions for crystal growth either by temperature shift or detergent exchange.
The results of this systematic work are currently being applied to other membrane proteins like PhoE, LamB, bacteriorhodopsin, as well as eukaryotic membrane receptors and will be useful for future work in the field.

BIO 366

INOSINE IS AN ADRENERGIC NEUROTROPHIC SUBSTANCE FOR CULTURED CHICK SYMPATHETIC NEURONS

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A large body of evidence now exists to support the participation of environmental signals in regulating transmitter phenotypic expression. We have observed previously that liver-conditioned-medium (LCM) contains a low molecular weight component which promotes metabolism and adrenergic, but not cholinergic development in cultured chick sympathetic neurons. We report here that this substance is inosine, the deaminated metabolite of adenosine. Indeed, analysis of the low molecular weight fraction of LCM by HPLC shows that the neurotrophic activity co-elutes with, and has the same absorption spectrum as inosine. Inosine increases neuronal metabolism and catecholamine, but not acetylcholine production by the sympathetic neurons in a dose-dependent fashion with a half-maximal stimulation at a concentration of 10^{-6} M. This effect can be blocked by 5 x 10^{-6} M dipyridamole, a nucleoside uptake inhibitor. This is the first evidence that a purine metabolite, i.e. inosine, can modulate the adrenergic properties of cultured sympathetic neurons.

BIO 367

CLONING AND EXPRESSION OF THE CATALYTIC SUBUNIT OF THE TYPE 2A PROTEIN PHOSPHATASE

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Protein phosphatases play a central role in the hormonal regulation of cellular metabolism. However very little information is available on the genetic complexity of these important regulatory enzymes. Protein phosphatase 2A exists as three different isotypes, termed $2A_o$, $2A_1$, and $2A_2$. All these forms are composed of a catalytic (C) subunit associated with different regulatory subunits. We have isolated cDNAs encoding the human and porcine C subunit. DNA sequence analysis revealed that two different classes of cDNA code for the C subunit, designated C_α and C_β. The two cDNAs code for very similar, but distinct polypeptides. Oligonucleotide probes specific for the C_α and C_β showed that both types of C subunit were encoded by 2kb mRNAs. Southern analysis confirmed that the two forms of C subunit were products of distinct genes. Analysis of the expression of C_α and C_β mRNAs showed that they are expressed in a tissue specific manner. The physiological regulation of phosphatase expression is currently being investigated.

BIO 368

RATE LIMITING REACTIONS OF THE RESPIRATORY BURST IN HUMAN NEUTROPHILS.

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The respiratory burst (RB) can be induced in human neutrophils with the chemotactic peptide fMLP or with platelet activating factor (PAF). Luminol dependent chemiluminescence measurements reflecting the activity of the burst enzyme (NADPH oxidase) show that the onset of the RB is identical for both agonists but the extent is much larger with fMLP. The signal transduction consists of at least two parallel sequences of reactions which act in concert to activate the oxidase. Studies of the lag preceding the onset of the burst reveal that one sequence is fast and the other is rate limiting. The rate limiting one is responsible for Ca release and can therefore be assigned to the sequence involving IP_3 and DAG formation and the subsequent activation of protein kinase C. In neutrophils prestimulated with PAF and restimulated 10 to 60 sec later with fMLP we observed a transient shortening of the lag. This indicates that at least one of the steps in the rate limiting sequence of reactions is only transient and ends long before the termination of the burst.

BIO 369

LONG-TERM STIMULATION OF cAMP PRODUCTION IN LLC-PK1 CELLS BY A PHOTOACTIVATABLE ANALOGUE OF VASOPRESSIN.

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A photoreactive analogue of vasopressin, 1-(3-mercapto) propionic acid, 8-(N^6-4-azidophenylamidino)Lys-vasopressin, was compared to [8-Arg]-vasopressin with respect to stimulation of cAMP synthesis in the LLC-PK1 pig kidney epithelial cell line. Without photoactivation, the vasopressin analogue-elicited responses were identical to those induced by vasopressin, in that cAMP synthesis returned to the basal, unstimulated level about 4 h after hormonal treatment. When activated by ultraviolet irradiation, the vasopressin analogue induced "permanent" stimulation of adenylate cyclase, whereby cAMP production could be detected even 12.5 h after treatment. In contrast to vasopressin or the nonactivated analogue, the photoactivated analogue inhibited growth of LLC-PK1 cells, apparently as a consequence of the prolonged stimulation of adenylate cyclase. The results imply that the cAMP-PK is the mediator of the hormone-stimulated growth inhibition.

BIO 370

CHARACTERIZATION OF THE INTRACELLULAR CALCIUM POOL SENSITIVE TO INOSITOL 1,4,5-TRISPHOSPHATE IN ADRENAL CORTICAL CELLS

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The steroidogenic response of adrenal cortical cells to angiotensin II is mediated by a transient rise of cytosolic free calcium concentration. This Ca^{++} is partly mobilized from an intracellular pool sensitive to inositol 1,4,5-trisphosphate ($InsP_3$). Previous studies in permeabilized cells have shown that this pool is vesicular and nonmitochondrial. This pool released Ca^{++} in response to $InsP_3$ in a concentration-dependent manner (EC_{50} = 0.6 uM). Further characterization of the $InsP_3$-sensitive pool was achieved by subcellular fractionation. The postmitochondrial supernatant was centrifuged at 25'000 x g for 20 min and the pellet was then fractionated on a discontinuous sucrose gradient. The specific binding of [^{3}H]$InsP_3$ was measured in the various fractions. $InsP_3$-induced Ca^{++} release was correlated with the binding capacity. The tracer was not metabolized during the binding assay. A sucrose gradient was developped for one-step separation of the $InsP_3$ binding site from the plasma membrane (PM) and the endoplasmic reticulum (ER). The specific binding was inversely correlated with the sulfatase C activity and the membrane bound RNA, two markers of ER. In conclusion, the organelle releasing Ca^{++} upon binding of $InsP_3$ in adrenal cortical cells is distinct from the ER and the PM.

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SUBCELLULAR LOCALIZATION AND KINETIC PROPERTIES OF PHOSPHATIDYLINOSITOL 4,5-BISPHOSPHATE PHOSPHOLIPASE C AND INOSITOL PHOSPHATES ENZYMES FROM HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS

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Human non-stimulated peripheral blood mononuclear cells exhibited phosphatidylinositol 4,5-bisphosphate phospholipase C (PIP_2-PLC), inositol 1-monophosphate (IP) and inositol 1,4,5-trisphosphate (IP_3)-phosphatase activities. By analytical subcellular fractionation it was found that these enzymatic activities were mainly recovered in the cytosol fraction (60-70% of the total activity) when compared to conventional plasma membrane marker enzymes. Concerning the kinetic parameters, PIP_2-PLC displayed the highest activity at pH 5,6 and pH 6,0 for cytosol and particulate fractions respectively, whereas IP and IP_3-phosphatases showed a pH_o at 7,0 in both fractions. While the PIP_2-PLC displayed close apparent Km values in cytosol and particulate fractions, both phosphatases were found to show substrate affinities for IP and IP_3 characteristic of these two fractions with a significant higher affinity (low Km) in the soluble fraction. In addition lithium was found to be a non-competitive inhibitor of the IP-phosphatase.

BIO 372

ROLE OF PROTEIN KINASE C IN HOMOLOGOUS DESENSITIZATION OF ANGIOTENSIN II-INDUCED PHOSPHOINOSITIDE HYDROLYSIS

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Rat mesangial cells respond to a challenge with angiotensin II with activation of a phospholipase C and calcium mobilization [Pfeilschifter & Bauer (1986) Biochem. J. 236, 289-294]. Pretreatment of cells with angiotensin II, followed by removal of the hormone, resulted in a decreased response to a second application of angiotension II. Pretreatment with angiotensin II had no effect on subsequent responsiveness to vasopressin. The specific antagonist saralasin did not inhibit the response to a subsequent stimulation with angiotensin II. After angiotensin II pretreatment, a prolonged incubation (120 min) restored responsiveness of the cells to angiotensin II. Pretreatment of mesangial cells with inhibitors of protein kinase C almost completely attenuated the desensitization of angiotensin II-stimulated IP_3 generation. These results indicate that, angiotensin II induces a homologous desensitization of phospholipase C stimulation. It is proposed that protein kinase C activation plays an important role in the molecular mechanism of desensitization of angiotensin II-stimulated polyphosphoinositide metabolism.

BIO 373

OVEREXPRESSION OF α-PKC LEADS TO THE SUPERINDUCTION OF THE uPA GENE EXPRESSION IN LLC-PK1 CELLS

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Phorbol esters such as TPA are known to induce the expression of the urokinase-type plasminogen activator (uPA) in LLC-PK1 cells. To characterize the role of PKC (the major phorbol ester receptor) in the uPA synthesis, the porcine LLC-PK1 cells were transfected with a mammalian expression vector (pZEM-3) carrying the α-type PKC in the presence of a selectable gene marker (pSV2-Neo). Cells were screened for overexpression of PKC by phorbol ester binding. Four out of 60 cell clones were positive and were further characterized by Western-, Northern and Southern blot analysis. Overexpression of PKC activity from 3 to 20 times were observed and roughly correlated with elevated levels of α-type PKC m-RNA. Transcription of uPA-mRNA was found to be superinduced (3-5-fold) following PKC activation by TPA of stably transfected as compared to the non transfected LLC-PK1 cells. These data indicate that superinduction of the uPA gene may be a consequence of PKC overexpression due to an altered phosphorylation state of the putative transacting factor AP-1.

BIO 374

PLASMA MEMBRANE DEHYDROGENASES IN RAT BRAIN SYNAPTIC MEMBRANES

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Trans plasma membrane dehydrogenases play a significant role in energy transduction and post-receptor signal transmission of most cells investigated. In neuronal tissues, the NADH-dehydrogenases were shown to be modulated by various neurotransmitters. We have further investigated the dopamine-sensitive NADH-dehydrogenases in rat brain synaptic plasma membranes. Three enzymes complexes could be separated electrophoretically under native conditions from detergent-treated synaptic membranes. The kinetics of the NADH-dehydrogenases was investigated and exhibits unusual complexity with respect to both substrates. NADH inhibits over 0.150 mM, and the electron acceptor (ferricyanide) displays a biphasic behavior with sudden inflexion at ca. 3 mM., which can be interpreted either in terms of enzyme aggregation or as reflecting cooperative effects. These data reflect the dramatic sensitivity of synaptic membranes towards the redox environment. The influence of neurotransmitters on kinetical parameters of NADH-dehydrogenases is also presented.

BIO 375

ACETYLCHOLINESTERASE IN A MOUSE NEUROBLASTOMA CELL LINE

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Cell associated and secreted AChE from the mouse neuroblastoma cell line NB_2A was partially purified by affinity chromatography and analyzed for amphiphilic properties. Cell extracts contained predominantly amphiphilic monomeric AChE and minor amounts of amphiphilic as well as hydrophilic tetrameric AChE. In contrast the culture supernatant only contained the hydrophilic tetrameric form. By digestion with proteinase K but not with PI-specific phospholipase C, amphiphilic AChE was converted to a hydrophilic form. SDS-gel electrophoresis of [^{3}H]-DFP-labelled AChE gave one band at 64 kD under reducing conditions and two additional bands at 120 kD and 140 kD under nonreducing conditions. These results indicate that the molecular weight of the catalytic subunit is 64 kD for monomeric and tetrameric AChE. However, tetrameric AChE seems to be composed of a light (120 kD) and a heavy dimer (140 kD) of catalytic subunits. In addition the heavy dimer contains probably a 20 kD subunit similar to that of human and bovine brain AChE which is thought to anchor the enzyme to the membrane.

BIO 376

DEPOLARIZATION-COUPLED CAPACITANCE CHANGES IN CULUTURED BOVINE ADRENAL CHROMAFFIN CELLS

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We have measured changes in membrane area by monitoring cell membrane capacitance with a patch clamp amplifier and a lock-in amplifier (Neher, E.;Marty, A.; PNAS 79, 6712-6716 (1982)) in order to quantifiy exocytotic activity in single bovine adrenal chromaffin cells. Depolarizing pulses of 10ms to 50ms from a holding potential of -70mV activate voltage dependent calcium channels and produce a fast jump increase in the capacitance signal. The magnitude of this jump depends at least in part on the amount of calcium that enters during the pulse. Thus the capacitance increase was largest for depolarizations to around +20mV, which activate the peak calcium current and was reversibly blocked by substitution of magnesium for calcium . A second, slower phase of capacitance increase could be observed with either repetitive or longer (100ms-200ms) depolarizing pulses. Investigation of the different cellular processes involved in the fast vs. the slow capacitance increases should provide information about molecular mechanisms underlying stimulated secretion.

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BIO 377

ENGINEERING OF ANTI-MMTV SECRETORY IGA

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Mouse mammary tumor virus (MMTV) induces mammary tumors in female mice of certain strains. The infection is transmitted from infected mother to newborns via milk. Monoclonal antibodies (MB 2.2) have been raised against MMTV and recognize a continous epitope on the envelope protein gp52. A strategy using fused proteins has been defined to identify this epitope.
In order to test whether monoclonal antibodies have protective properties, they have to be introduced in the gastrointestinal tract of the suckling mice, which requires stabilization by binding to secretory component (SC). This latter protein is the cleaved part of the polymeric immunoglobulin receptor (pIgR), responsible for the uptake of dimeric IgA and their transport across mucosal epithelia. In order to obtain SC, SP20 myeloma cells as well as MB 2.2-producing hybridoma cells were transfected with a cDNA coding for rabbit pIgR and expression at the protein level has been analyzed. It becomes possible to test whether SC protects IgA from proteolytic degradation.

BIO 378

STRUCTURE AND BIOLOGICAL PROPERTIES OF A NOVEL NEUTROPHIL-ACTIVATING FACTOR (NAF)

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NAF, a polypeptide produced by human monocytes stimulated with LPS, ConA or PHA, was purified to homogeneity and its effects on human neutrophils were studied. Amino acid sequencing led so far to the identification of 32 out of 50-60 presumed residues (S A K E L R C Q C I K T Y S K P F H P K F I K E L R V I E S G P) and showed that NAF is a novel peptide with no significant homology to IL-1, TNF, IFNs, CSFs and C5a. NAF elicits the respiratory burst and granule exocytosis in human neutrophils by a receptor-mediated process that is accompanied by a transient rise in cytosolic free calcium. The respiratory burst response is similar in onset and time course to that induced by C5a or fMLP. NAF-stimulated neutrophils become desensitized to NAF, but respond normally to C5a, fMLP, PAF and LTB4, indicating that a distinct receptor exists for this novel peptide. In its function, NAF appears homologous to C5a, the other host-derived neutrophil-activating peptide. Unlike C5a, however, NAF is not inactivated be serum peptidases and thus presumably accumulates in inflamed tissues.

BIO 379

INTERACTION OF THE CYTOSKELETAL PROTEIN VINCULIN WITH BILAYERS DEMONSTRATED IN INTACT CELLS

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Vinculin, a putative actin-plasma membrane linker, has been shown to insert in vitro into the hydrophobic core of the bilayer, using a photoactivatable lecithin-analogue. This process is critically dependent on the presence of acidic phospholipids. We now present evidence for such an interaction to occur also in intact chicken embryo fibroblasts. The cells were incubated for 2 hrs with a ^{3}H-labeled photoactivatable fatty acid, followed by photolysis, cell fractionation and immunoprecipitation of vinculin. Most of the label taken up by the cells co-purifies with membranes, as fatty acid, or incorporated into lipids. Vinculin is found both in the cytosolic and in the membrane fraction. However, only labeling of membrane associated vinculin is markedly increased by photolysis (3-5x). Moreover, upon photolysis the membrane-bound vinculin contains about 14x more label than the cytosolic vinculin. These results suggest, that the interaction of vinculin with acidic phospholipids, observed in vitro, may also be relevant in intact cells, and may enable the protein to act as an actin-membrane linker in areas of cell-substrate and cell-cell contacts.

BIO 380

THE CYTOSKELETON OF TRYPANOSOMA BRUCEI

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The cytoskeleton of the parasitic hemoflagellate Trypanosoma brucei contains three major structural components: i) the membrane skeleton consisting of a dense layer of singlet microtubules ii) the flagellar axoneme and iii) the paraflagellar rod (PFR), associated to the axoneme.
One abundant cytoskeletal protein (p60) was found to bind in vitro both to microtubules and to membrane vesicles or liposomes. p60 can crosslink these structures in vitro and hence may function in vivo as a linker between microtubules and the cell membrane. We present the analysis of the coding sequence for p60 and discuss the possible functional domains.
The PFR is a highly ordered protein structure, extending along the entire length of the flagellum. It consists of two immunologically similar proteins of 68 and 70 kDa. Their biochemical behaviour as well as the amino acid composition suggest a similarity with intermediate filaments of metazoa. Polyclonal antibodies were used to isolate the corresponding genes, and sequencing will give us definitive information about the relation to intermediate filaments.

BIO 381

MODULATION OF GELSOLIN CONTENT IN TUMORAL CONDITIONS

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Gelsolin regulates actin filament length by severing, capping and nucleating actin filaments. In tumor cells, increased amounts of F-actin without a change in its total amount indicate a reorganization in the state of the actin. The presence of gelsolin in a large variety of mammalian cells favors its role as an actin modulator. This prompted us to compare the expression of gelsolin in normal and malignant tissues. By immunohistochemistry we have localized gelsolin, actin and prekeratin in frozen sections of normal human breast and canalicular carcinomas. A positive staining for gelsolin was observed in the epithelial cells, in the myoepithelial cells surrounding the gland and in the stromal fibroblasts of the normal mammary gland. In contrast, no staining for gelsolin was detectable in carcinomatous cells, although myofibroblasts of the stroma, remaining myoepithelial cells still displayed a high positivity for gelsolin. This absence of gelsolin staining in the breast carcinoma may reflect dedifferentiation, as well as proliferative or invasive activities of tumoral cells. The immunohistochemical detection of gelsolin may be a useful adjunct for the study of mammary gland epithelium malignant transformation.

BIO 382

STUDIES ON THE MECHANISMS OF HMPS STIMULATION AND PARASITE DESTRUCTION BY MACROPHAGES EXPOSED TO ELECTRON CARRIERS

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Exposure of macrophages (Mø) to electron carriers (EC) such as methylene blue (MB) stimulates the hexosemonophosphate shunt (HMPS) of the cells and their capacity to kill intracellular Leishmania parasites. The mechanisms of these effects were studied in cell-free systems. Addition of MB to NADPH led to O_2 consumption and parasite killing in the reaction mixture. Cytochrome c, a scavenger of superoxide (O_2^-), abolished both O_2 consumption and parasite destruction. Nitroblue tetrazolium reduction by the mixture was inhibited by superoxide dismutase, pointing to O_2^- production. Catalase also protected the parasite, suggesting that one toxic factor generated in the mixture was H_2O_2. MB-dependent HMPS stimulation was further studied in a mixture composed of all substrates, enzymes and cofactors of the HMPS. MB again stimulated O_2 consumption and parasite killing. Cytochrome c but not catalase inhibited the latter effect, suggesting that molecular species other than O_2 metabolites might also be involved in microbicidal activity under these in vitro conditions, and presumably in macrophages exposed to EC.

BIO 383

CHARACTERIZATION OF THE END1 GENE REQUIRED FOR ENDOCYTOSIS IN YEAST

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The yeast endocytosis mutant end1 does not accumulate the fluorescent endocytic marker Lucifer Yellow CH and is defective both for uptake of pheromone and the late pheromone response. In addition we have found that end1 has no apparent vacuole, is defective in vacuolar enzyme processing, is unable to grow on nonfermentable carbon sources while respiration is normal, and has an elevated kinase activity. Recently it has been shown that this mutant was unable to express immunity to type 1 killer toxin (Sturley, S.L. and Bostian, K.A.). Null end1 mutants (END1 gene disrupted or deleted) are viable and display a phenotype which resembles the original mutant. The END1 gene has been cloned and DNA sequence analysis revealed that the gene codes for a 117 kDa protein containing an ATP binding domain homologous to one described for several enzymes (ATPases). Interestingly the END1 gene codes for two rather rare RNA transcripts which are not present in the end1 null mutant.

BIO 384

EPITOPE MAPPING OF TETANUS TOXIN SPECIFIC HUMAN B AND T HELPER LYMPHOCYTES

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Epitopes recognized by tetanus toxin specific B cell lines and T helper cell clones were mapped. Three of four B cell lines examined were specific for the tetanus toxin C-fragment whereas the fourth recognized a site located on the B-fragment. The five characterized T cell clones displayed a different antigen specificity. Two clones recognized sites on the tetanus toxin C-fragment, one of which is located within the peptide formed by residues 1273-1284 of tetanus toxin. The three other clones were specific to the B-fragment. MHC class II restriction of these five clones was examined. These clones were DR restricted, two of them being DR 3 restricted and the three others DR5 restricted.

BIO 385

BASIC DIFFERENCES BETWEEN CHICKEN AND MAMMALIAN MUSCLES

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We have determined the fiber type composition of seven major chicken thigh muscles with the histochemical methods myofibrillar ATPase, α-Glycerolphosphatedehydrogenase (glycolytic capacity) and NADH-Tetrazoliumreductase (oxidative capacity). Chicken muscles are composed principally of a mixture of type II (fast twich) and type III (slow tonic) fibres. This is in contrast to mammalian muscles, which are composed of type II (fast twich) and type I (slow twich) fibres, while type III fibres are only found in extraocular muscles. In chicken, however, type I fibres are rare, they were found only in m. sartorius and m. adductor medialis.

Chicken type III fibres have a high oxidative capacity, but display varying glycolytic capacities: In muscles with many type III fibres, their glycolytic capacities are low, while in muscles with few type III fibres, they are rich in glycolytic enzymes. These results suggest that in chicken, type III fibres take the place of type I fibres in mammalian muscle.

BIO 386

A PROTEIN SECRETED BY HORMONE INDEPENDENT HUMAN MAMMARY TUMOR CELL LINES IS A FAST ACTING SPREADING FACTOR FOR HORMONE DEPENDENT CELL LINES

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The estradiol receptor-lacking human mammary cell lines MDA-MB-231 and HBL-100 both secrete a protein which induces a quick spreading of estradiol receptor-containing cell lines MCF-7, ZR-75-1 and T47-D. First sign of spreading activity can be observed as early as 10 minutes after addition of conditioned media. After a few hours, the intercellular contacts are loosened, the cells start to make protrusions and separate. Normal morphology is reassumed only after several days following factor removal. Similar but slightly slower effects are induced on the same cells by tumor promoter 12-O-Tetradecanoylphorbol 13-Acetate. But unlike tumor promoters, the spreading factor does not activate protein kinase C. Size exclusion chromatography with a Superose 12 column revealed a molecular mass of approx. 65,000. Preliminary experiments with the partially purified factor suggest that it is a growth inhibitor for estradiol receptor-positive human mammary tumor cell lines.

BIO 387

OSCILLATIONS OF RESPIRATORY BURST AND SHAPE CHANGE IN HUMAN NEUTROPHILS IMPLICATIONS FOR THE CONTROL OF THE TWO CELL RESPONSES

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Neutrophils stimulated with chemoattractants (N-formyl-Met-Leu-Phe, complement factor C5a, platelet activating factor and leukotriene B_4) showed a transient increase in on-going respiratory burst activity following prestimulation with nM concentrations of 12-phorbol,13-myristate acetate (PMA) or 1-oleoyl,2-acetyl glycerol (OAG). Low agonist concentrations or the presence of a secretion inhibitor (17-hydroxywortmannin) led to the development of identical, temperature-dependent oscillations (period 7.9 s at 37°C) which coincided with turbidimetrically-detected fluctuations in apparent cell shape. Thus it seems that changes in shape and in respiratory burst enzyme (NADPH-oxidase) activity are either controlled by common features of the cell's transduction mechanism or the morphological alterations directly affect NADPH-oxidase activity. The shape oscillations were not inhibited by chelating intracellular calcium with high concentrations of QUIN-2, suggesting that increases in internal Ca^{2+} concentration were not important for control of the oscillations. While PMA was necessary to restore NADPH-oxidase activity in cells with lowered Ca^{2+} levels, protein kinase C seemed not to be required for shape changes. The use of antagonists revealed a strict need for continous receptor occupation by agonists and indicated the importance of the ligand-receptor complex in the regulation of the oscillatory phenomenon.

BIO 388

OSCILLATORY MOTION OF HUMAN NEUTROPHILS: KINETIC MODELLING AND ANALYSIS OF TURBIDIMETRIC PROGRESS CURVES BY LIGHT SCATTERING THEORY

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Human neutrophils in suspension pretreated with a secretion inhibitor (17-hydroxywortmannin) show transient and oscillatory turbidimetric progress curves upon stimulation with chemotactic factors as N-formyl-Met-Leu-Phe, complement factor C5a, leukotriene B_4 or platelet activating factor. Fitting the shape change response curves with a modified ABC series kinetic model yields the rates of interconversion of the kinetically-distinguishable morphological forms and their light scattering extinction coefficients. The mean response was characterized by a single rate constant ($0.18\ s^{-1}$), revealing that a rate limiting step controls the overall shape change induced by all four stimuli. The mean frequency of the shape oscillations was similar ($0.13\ s^{-1}$).

The fitted extinction coefficients of four kinetically-distinct neutrophil forms could be closely reproduced by light scattering theory calculations based on simple geometrical models of cell size and shape. Rythmic extension and partial retraction of lamellopodia periodically decrease and increase the cell body volume, with a maximal decrease of about 25% and a final decrease of ca. 10% of the total cell volume. The shape oscillations appear to be related to a swimming or crawling motion, which is required for migration to infection sites.

BIO 389

T4 regulation of the actin-cytoskeleton in astrocytes.

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DbcAMP stimulation of cultured astrocytes induces 5'-deiodinase type II (5'D-II), catalyzing intracerebral conversion of T4 to T3. Turnover of 5'D-II is rapid, T4-dependent, and cytochalasin blocks its degradation. We examined the interactions between the actin-cytoskeleton and 5'D-II. Astrocytes plated at different initial density, were stimulated for 16h +/- FCS. In both FCS and FCS-free cultures, the half-life of 5'D-II increased from 9.7 to 17.6 min, and 75 to 288 min, respectively with increasing cell plating density. SDS-PAGE profile of cytoskeletal (Triton-insoluble) proteins showed that increasing cell density plating caused a progressive diminution in the cellular content of actin. In FCS-free cultures, the F-actin content was 37% decreased compared to FCS cultures. T4 (10 nM/16h) restored the F-actin content. In hypothyroid cultures, 5'D-II activity increased with an increase in the half-life and a decrease in the actin content; this process can be reversed by T4. Similar changes in 5'D-II levels, half-life and F-actin content were obtained with increasing initial cell plating density. These data suggest a close relationship between the polymerization state of actin and the degradation/inactivation of the 5'D-II.

BIO 390

Reverse T3 inhibition of cerebellar 5'D-II

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The role of local T4 to T3 conversion in the cerebellum of new born rats was tested by the ability of rT3 to inhibit cerebellar 5'-deiodinase type II (5'D-II), catalyzing intracellular conversion of T4 to T3. Neonatal hypothyroid rats (n=5/group) were injected twice daily s.c. (day 5 to 7) with different doses of rT3. Cerebellar 5'D-II activity was measured according to Leonard and Rosenberg (1 nM rT3, 10 mM DTT, 1 mM PTU). 5'D-II activity in controls was 34.26 ± 8.92 fmol/h/mg protein. With 100 ug rT3/100 g BW, 5'D-II was 6.44 ± 1.24 and with 200 ug rT3, 3.85 ± 0.57 fmol/h/mg protein, which correspond to maximal inhibition (88.8%). Cerebellar conversion of T4 to T3 was measured 3, 6, 12 and 20 h after an i.v. injection containing 3.24 uCi/animal [^{125}I]T4 and 0.2 ug T4/animal ± 20 ug rT3/rat. In the control animals (n=6) the cerebellar T3/T4 ratio was 3.1% after 3h, 4.1% after 6h, 7.3 % after 12h, 9.3% after 20h. Under rT3, the ratio was 1.6% to 2.8% with no consistent change in time. Disappearance of serum T4 or T3 was not modified by rT3 injections. We conclude that in the neonates 5'D-II in cerebellum can be efficiently inhibited by rT3. This treatment inhibited the local conversion by a factor of 3.

BIO 391

MODULATION OF CULTURE CONDITIONS PROMOTES HYPERTROPHIC OR REST CARTILAGE PHENOTYPE OF CHONDROCYTES

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Cartilage cells are morphologically diverse depending on their location in the tissue. Cells of rest cartilage show a low tendency to proliferate, are relatively small, and synthesize collagens II, IX, and XI. Cells from calcifying cartilage undergoing endochondral ossification are hypertrophic and synthesize also large amounts of collagen X.
Chick embryo cartilage cells were cultured in agarose. Conditions were established under which cells developed a specific phenotype and deposited extracellular matrix, accordingly. At low density, cells required high serum concentration, became hypertrophic, and synthesized collagen X. At high density, cells had no serum requirement, resembled rest cartilage cells and synthesized little if any collagen X. The extracellular matrix contained collagen fibrils similar to those of authentic cartilage.
We conclude, that serum factors promote hypertrophy and that cartilage cells secrete other factor(s) favoring expression of rest cartilage phenotype.

BIO 392

REGULATION OF ADENYLATE CYCLASE AND α-2 ADRENOCEPTORS DURING REVERSIBLE PLATELET ACTIVATION

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Washed platelets in the absence of physiological activators possess gravity inducible shape change. This is paralleled by an increase of phosphatidic acid (PA), polyphosphoinositides (PPI) and an inhibition of PGE1- and Gpp(NH)p-stimulated adenylate cyclase (AC) activity. Incubation of platelets at 37°C for 1 hour decreases [32P]PA and [32P]PPI and restores their response to a low dose of thrombin (0.015 U/ml). Simultaneously an increase of PGE1- and Gpp(NH)p-stimulation of AC is observed. The relaxation of the platelets influences predominantly the cAMP levels without significantly affecting the dissociation constants of the stimulators. Forskolin-induced activation of AC does not differ between stimulated and relaxed platelets. It is suggested that the initial increase of PA inhibits the coupling of regulatory and receptor proteins to AC and has no effect on the catalytic unit. In addition a decrease of the ability of Gpp(NH)p to modulate α-2 adrenoceptors and a reduction of the affinity of epinephrine for [3H]yohimbin binding sites is found. Relaxed platelets restore the regulation of α-2 adrenoceptors by guanine nucleotide.

BIO 393

IMPAIRMENT OF MACROPHAGE COLONY-STIMULATING FACTOR (M-CSF) PRODUCTION IN THE OSTEOPETROTIC op/op MOUSE

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M-CSF induces the proliferation and differentiation into macrophages from committed progenitor bone marrow cells. Furthermore, it might also be necessary for the recruitment of osteoclasts. Previously we have shown that osteoblasts produce M-CSF. It has been suggested that in the osteopetrotic op/op mouse the formation of M-CSF may be impaired (Wiktor-Jedrzejczak, J.Exp.Med. 156:1516, 1982). To test this hypothesis, bone tissue (calvaria) from 10 day old mice was incubated for 3 days in BGJ_b medium. M-CSF released into the medium was measured and identified with antiserum against M-CSF (gift of Dr. E.R. Stanley, Bronx, NY). Whereas the calvaria from control animals (+/?) synthesized M-CSF, those from op/op mice did not. A concomitant production of a possible inhibitor of M-CSF activity could be excluded. The data suggest that M-CSF may be required for osteoclast formation and hence the lack of this factor might be a cause for certain types of osteopetrosis.

BIO 394

CELLULAR LOCALIZATION OF CALBINDIN D-28 mRNA USING IN SITU HYBRIDIZATION HISTOCHEMISTRY: VITAMIN D DEPENDENCE OF THE BRAIN CALBINDIN D-28 ?

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The cells expressing the Calbindin D-28 (the 28 KDa Vit.D dependent Calcium Binding Protein) have been localized in rat tissues by in situ hybridization histochemistry using ^{35}S-labelled RNA probes complementary to the rat Calbindin mRNA sequence. Distal tubule cells in kidney and Purkinje cells in cerebellum contain high amount of Calbindin mRNA. Granule cells of the dentate gyrus exhibit also a specific staining as well as pyramidal cells of the hippocampus but especially in the lateral part of this region. Caudate-putamen, superficial layers of cerebral cortex, reticular thalamic nucleus, hypothalamus and substantia nigra compacta are also stained. We are studying how the brain Calbindin D-28 is regulated especially by vitamin D and whether a cell specific regulation occurs, refering to the fact that vit.D receptor do not appear in all Calbindin D-28 positive cells.

BIO 395

ACTIVATION OF THE T CELL LINE PC60 WITH INTERLEUKIN-1: MODULATION BY CYCLIC AMP.

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The stimulation of the T cell line PC60 by interleukin-1 (IL-1) to produce the serine esterase, BLT esterase (BLTE), is enhanced by the stimulation of the adenylate cyclase or by the addition of derivatives of cyclic AMP (cAMP). Earliest BLTE is detected 12 h after IL-1 addition and maximal BLTE activity is found three days after stimulation. cAMP added simultaneously with IL-1 has a synergistic effect on the stimulation by IL-1, it does not accelerate the response to IL-1 nor does it change the affinity of the cells for IL-1. cAMP alone has only a minor stimulatory effect. In addition to the enhancing effect which is maximal when cAMP was added 12 h after IL-1, we found an inhibitory effect of dibutyryl cAMP when it was added prior to IL-1. The inhibition was larger than 85% after a preincubation period of 16 h and it was not due to butyrate as shown with NaBt added as a control.

BIO 396

STIMULATION OF THE EXPRESSION OF THE RECEPTOR FOR INTERLEUKIN-2 BY INTERLEUKIN-1 IS ENHANCED SYNERGISTICALLY BY CYCLIC AMP.

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The expression of interleukin-2 receptors (IL-2R) in PC60 cells, a murine T cell hybridoma, is induced by interleukin-1 (IL-1) and enhanced by interleukin-2 (IL-2).
Here we show that the IL-1 effect is also enhanced by derivatives of cyclic AMP (cAMP) such as dibutyryl cAMP or 8BrcAMP. cAMP alone does not induce the IL-2R. Since IL-2 enhances the expression of IL-2R due to IL-1 but does not markedly increase the level of cellular cAMP, we think that cAMP acts in a different way on the cells than does IL-2. cAMP plays a similar role in the induction of a granular serine esterase (BLT esterase) and therefore seems to facilitate induction due to IL-1 in a general way.

BIO 397

PARTIALLY DEGRADED INSULIN IN THE CIRCULATION OF RATS INJECTED SUBCUTANEOUSLY WITH SEMISYNTHETIC TRITIATED INSULINS

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Tracer studies with radio-iodinated insulins have indicated that partially degraded forms of insulin may be released from physiological sites of insulin degradation and re-enter the circulation. The chemical nature of such fragments and their physiological importance have not been determined.

We have used semisynthetic tritiated insulins injected subcutaneously into rats. In plasma prepared 60 minutes after injection, we observe the presence of radioactivity eluting just after insulin by gel filtration, whether the label is present at the amino-terminus of the A or B chain. Such material is not observed in controls where the blood from rats injected with saline is collected into tubes containing the tritiated insulin. In addition, the radioactive material eluting just after insulin binds less well to anti-insulin Sepharose than the radioactivity eluting in the position of insulin. Further characterization is in progress.

BIO 398

(Na,K)ATPase OF PERIFUSED RAT HEPATOCYTES IS STIMULATED IN RESPONSE TO EXOGENOUS Ca^{2+}

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Ca^{2+} mobilisation and K^+ uptake preceed the metabolic responses of hepatocytes to Ca^{2+}-dependent hormones. To answer the question whether K^+ uptake is mediated by Ca^{2+} we have studied the effect of exogenous Ca^{2+} in the presence of vasopressin (10 nm). An increase of extracellular Ca^{2+} (10 µM to 1.3 mM) produced a transient uptake of K^+ (7.4 nmol/mg protein) and an enhanced glucose and lactate production reflecting an activation of glycogenolysis. Ouabain (0.8 mM) inhibited the Ca^{2+}-induced K^+ uptake by 90%, lactate production, H^+ release and O_2 uptake by 40%. Amiloride (0.1 mM) had no measurable effect, suggesting that stimulation of (Na,K)ATPase was not secondary to an increased intracellular Na^+ concentration due to Na^+/H^+ exchange. Ca^{2+} may activate (Na,K)ATPase by altering the environment of the enzyme within the membrane or indirectly by increasing the availability of glycolytic ATP.

BIO 399

MONOCLONAL ANTIBODIES TO BOVINE DOPAMINE-BETA-HYDROXYLASE

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Dopamine-beta-hydroxylase (DBH), an enzyme of the catecholamine metabolism, catalyzes the conversion of dopamine to noradrenaline. The enzyme is located in the adrenal medulla and in noradrenergic nerve cells, and is also found in tumors of sympathoadrenomedullary origin. In the case of neuroblastomas, an early detection is essential due to the poor prognosis of the disease. Thus, MAb which selectively recognize DBH would prove useful as a diagnostic tool. Since human tumors are not readily available, the enzyme was isolated from bovine adrenal medulla. Polyclonal antibodies raised in rabbits also recognized purified human DBH, as confirmed by the immunoblotting technique. Thus, BALB/c mice were immunized with 10 µg of the bovine DBH, and MAb were produced by the hybridoma technique. The supernatants were screened by ELISA, also checking for reactivity with human DBH. Among the positive hybridomas, 4 bovine specific MAb were selected. One of them recognized DBH of both origin. The specificity of these MAb was further investigated.

BIO 400

ACTIVATION OF A T CELL LINE (PC60) DUE TO INTERLEUKIN-1 IS INHIBITED BY GLUCOCORTICOIDS.

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Interleukin-1 (IL-1) activates the murine T cell line PC60 to produce the serine esterase benzyllysylthioesterase (BLTE). This activation is enhanced by derivatives of cyclic AMP (cAMP) or by activators of the adenylate cyclase added simultaneously with IL-1.
Here we show that glucocorticoids (hydrocortisone and dexamethasone) are able to inhibit to more than 90% the induction of the BLTE due to IL-1 or due to the combination of IL-1 plus cAMP. The inhibition is dose dependent with 50% inhibition at 3.10^{-8} M for hydrocortisone. Glucocorticoids showed the full inhibitory effect if added up to 6 h after IL-1, but showed no inhibition if they were removed from the culture media after a preincubation prior to stimulation with IL-1.

BIO 401

BIOSYNTHESIS OF HUMAN INTESTINAL SUCRASE-ISOMALTASE (SI). N- AND O-GLYCOSYLATION.

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The biosynthesis and maturation of sucrase-isomaltase (SI) was investigated in cultured small intestinal biopsy specimens and mucosa explants. Pulse-chase experiments with ^{35}S-methionine revealed one endo H-sensitive intermediate of Mr = 210,000 (pro-SI_h) which was processed at a low rate to a complex glycosylated form of Mr = 245,000 (pro-SI_c). This latter form can be specifically and instantaneously (within 1 min) cleaved by trypsin to the two subunits isomaltase (I_c) and sucrase (S_c).

Enzymatic and chemical deglycosylation of SI with endo F and trifluoromethanesulfonic acid (TFMS) as well as chromatography of purified molecules on Helix pomatia lectin-Sepharose demonstrated that pro-SI_c, I_c and S_c are N- and O-glycosylated. Furthermore, the results were indicative of a posttranslational O-glycosylation of SI. Finally S_c, but not I_c, was shown to display several populations (at least four) varying in their content of O-linked glycans.

BIO 402

IN VITRO MODULATION OF GLYCAN CHAINS

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Heterologous expression of animal glycoproteins in yeast frequently yields products substituted with mannans which are not suitable for animal or human use. We devised a method of modulating these glycan chains by stepwise enzymatic treatments of yeast glycoproteins using purified Endoglycosidase H, galactosyl- and sialyltransferase in soluble phase systems or enzyme reactors and radioactive donor substrates. Incorporation of sialic acid was demonstrated by electrophoresis/autoradiography and susceptibility to cleavage by neuraminidase. In case of invertase as a yeast model glycoprotein, up to 50% of possible acceptor sites have been sialylated.
This work was partially supported by grant 3.013.084 of the Swiss National Science Foundation.

BIO 403

A cAMP-DEPENDENT POST-TRANSLATIONAL MODIFICATION OF SPECTRIN ALTERS ITS BINDING PROPERTIES

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Human red blood cell spectrin is almost completely extracted from membranes by low ionic strength buffers. The remaining membranes form vesicles (primarily of the inverted type) called IOVs, which retain ankyrin and a small fraction of both spectrin bands (IOV-associated spectrin). Unlike most ankyrin, IOV-associated spectrin is not extracted by high salt. The amount of IOV-associated spectrin is increased by incubating cells with 10-200 µM cAMP for 2 hrs or more. IOV-associated spectrin was selectively extracted from KCl-treated IOVs by urea at low ionic strength, indicating that urea-extractable spectrin is associated with the membrane by hydrophobic forces. Urea extractable spectrin from ^{32}P-labelled cells contains some phosphopeptides that are identical to those of a phosphoform of spectrin, which was generated by incubating spectrin dimer with γ-^{32}P-ATP and the catalytic subunit of cAMP-dependent protein kinase. This suggests that cAMP-dependent phosphorylation of spectrin renders it resistant to low ionic strength extraction and thus alters its binding properties.

BIO 404

ACYLATION OF MITOCHONDRIAL PROTEINS

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Mitochondria and mitoplasts from rat liver were incubated with [1-^{14}C] myristate, denaturated in boiling SDS and analyzed by SDS-PAGE and autoradiography. Six to eight protein bands were found to be labelled radioactively. If the mitochondria were heated to 95° C prior to incubation with this fatty acid, no labelling was observed. By preexposing the mitochondria to unlabelled fatty acids of varying chain lengths, the extent of labelling by [1-^{14}C] myristate was reduced in a chain length dependent manner, with a maximal effect at lauric acid. Reversibility of the labelling was demonstrated by chasing the incorporated radioactivity with unlabelled fatty acids of varying chain length, with a maximal displacement of the tracer at lauric acid. Fractionation of the mitochondria into mitochondrial matrix and inner mitochondrial membrane before or after labelling suggested a location of the modified proteins on the matrix side. After treatment of the labelled bands with hydroxylamine the recovered radioactivity comigrated with myristic acid on thin layer chromatography plates.

Supported by SNSF

BIO 405

EGASYN AFFECTS THE PROCESSING OF β-GLUCURONIDASE IN MOUSE LIVER

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Three differently modified forms of β-glucuronidase are identified: a microsomal form existing only in tissues where egasyn, a second microsomal protein is also present, an acidic (L_a) and a basic (L_b) lysosomal form occurring in all mouse tissues. L_b predominates in tissues containing microsomal β-glucuronidase, L_a in those lacking it. In pulse labelling experiments using strain C57BL/6 liver containing egasyn (Eg+/Eg+) and microsomal enzyme, about half of newly synthesized β-glucuronidase was processed to the microsomal form which evidently was further processed to L_b and approx. half directly to L_a. In liver of the congenic line C57BL/6.YBR Es-1^b Eg° lacking egasyn (Eg°/Eg°) and the microsomal enzyme, most of the labeled β-glucuronidase was processed to L_a, and only a minor portion to L_b. Labeled enzyme appeared first in a microsomal then in a light and finally in a heavy lysosomal fraction in Eg+/Eg+ liver. In Eg°/Eg° liver, no labeled enzyme was measurable in the microsomes, but it appeared rapidly in both types of lysosomes. Taken together these findings indicate that the microsomal form serves as precursor of L_b, and that L_a is synthesized independently. The apparent half life of L_a is only two thirds that of L_b; this fact accounts for the reduced β-glucuronidase activity in Eg°/Eg° liver which contains L_a as the predominant form.

BIO 406

IN VITRO ADDITION OF THE GLYCOLIPID ANCHOR TO THE THY-1 ANTIGEN

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The Thy-1 glycoprotein normally expressed at the cell surface of rodent thymocytes and neurons is anchored in the lipid bilayer via an inositol-containing phosphoglycolipid and can be released from the cell surface by phosphoinositol-specific phospholipases C (PI-PLC). The Thy-1 gene can code for a precursor protein of 162 amino acids including a N-terminal leader sequence of 19 amino acids and a C-terminal hydrophobic fragment of 31 amino acids. This hydrophobic segment is removed from the precursor shortly after synthesis and replaced by the phosphoglycolipid covalently attached to the amino acid cystein 112. Using a cell free system for protein synthesis, we demonstrate that addition of a phosphoglycolipid (glypiation) to the membrane protein Thy-1 can occur in vitro in the presence of rough microsomes. This system is now used to define which region of the precursor molecule is involved in the correct processing of the Thy-1 molecule.

BIO 407

BIOSYNTHESIS AND POSTTRANSLATIONAL MODIFICATIONS OF PROTEIN KINASE C IN HUMAN BREAST CANCER CELLS

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Several forms of protein kinase C (PKC) with different molecular weights (74/77 and 80 kD) were detected in subcellular fractions of human breast cancer cells by immunoprecipitation. Pulse-chase experiments with (^{35}S)methionine demonstrate that the pulse-labeled 74 kD PKC can be chased into the 77/80 kD PKC. A 74 kD protein was also generated by in vitro transcription-translation of the full length ↓PKC cDNA (obtained from P.J. Parker, Ludwig Institute, London) cloned into pSP65. Our data suggest that the 74 kD PKC may represent the precursor form of the 77/80 kD PKC. This 74 kD PKC precursor accumulates in human breast cancer cells when treated with phorbol 12-tetradecanoate 13-acetate (PTA) suggesting that the prolonged exposure of tumor promoters inhibits steps in the maturation to the 77/80 kD form. At least two posttranslational phosphorylation steps are required for the conversion of the putative 74 kD PKC precursor to the 77/80 kD form(s).

BIO 408

INFECTIVITY AND FUSOGENICITY OF SEMLIKI FOREST VIRUS (SFV) CONTAINING ALTERNATIVE GLYCANS

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The goal of this investigation was to study the effect of various glycosylated states of the SFV proteins on maturation and fusogenicity in Aedes albopictus cells and infectivity of the corresponding virions. Glycosidase inhibitors 1-deoxynojirimycin, deoxymannojirimycin and swainsonine had been shown to inhibit the formation of N-linked complex-type oligosaccharides. In Aedes albopictus cells treated with these inhibitors, release of virus particles was detected and the viral glycoproteins produced were Endo-H sensitive as shown by SDS-PAGE. Furthermore, in the presence of inhibitors, the infected cells were fusion competent and the virus produced was infectious. Thus we conclude that the oligosaccharides of viral glycoproteins play a minor role in the viral biological activity.

BIO 409

THE Au(I) ANTIRHEUMATIC COMPOUNDS INHIBIT PROTEIN KINASE C

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Purified protein kinase C (PKC) activated with Ca^{+2} and phosphatidylserine (PS) was inhibited in a concentration dependent manner by Au-S-glucose, Au-S-malate, and Auranofin (IC_{50} = 2.7, 3.8, 361 uM). The control compounds thiomalate and malate showed no inhibition of kinase activity indicating that the inhibition is specific to the Au(I) entity. Phorbol 12, 13 dibutyrate (PDB) - PS- actvated PKC was similarly inhibited in a concentration dependent manner in the micromolar range by the above Au(I) compounds. Reversal and preprotection experiments of Au(I) inactivated PKC with dithiothreitol, and the amino acids his and met indicate that the inhibition is not due to complexation of essential cys, met or his residues within PKC. Preliminary kinetic competition experiments indicate that AuATP is not the inactivating species.

BIO 410

PROTEIN KINASE C IS EXPRESSED IN HUMAN ADULT BRAIN

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Recently the complete nucleic acid and deduced amino acid sequences for protein kinase C (PKC) were reported by Coussens et al.(1986, Science 233, 859-866) from bovine and rat adult, and human fetal brain. Enzymatically active PKC however has not been reported in human adult or fetal brain. Here we report the expression of this gene in human adult brain. PKC was partially purified from human adult brain (post-mortem). In two chromatographic steps (Phenyl-sepharose and DE 52) kinase activity was purified 1790 fold relative to the 75% ammonium sulphate precipitate. The partially purified PKC had a specific activity in the presence of Ca^{+2} and phosphatidylserine of 17.9 nmol ^{32}Pi/min mg protein and a protein yield of 475 ug/500g tissue was obtained, both similar to those obtained from other tissues (Kikkawa, U. et al., 1983, Methods Enzymol. 99, 288-298; Schatzman, R.C. et al., 1983, Biochem. J. 209, 435-443).

BIO 411

IN PLASMA CELLS, ALTERNATIVE POLYADENYLATION CONTROLS THE LEVELS OF μ_s AND μ_m mRNA.

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The expression of different immunoglobulin μ and δ heavy chain mRNA species from the μ and δ complex transcription unit changes radically during B cell development. Post-transcription RNA processing controls the levels of secreted (μ_s) and membrane (μ_m) mRNA that differ only in their 3' termini. Alternative splicing or polyadenylation could be responsible for this differential processing. By deleting the region between the two poly (A) regions including the splice signals we demonstrate that the usage of the second poly (A) site (μ_m) is favored in plasma cells and in L cells. This result suggests that the choice of polyadenylation site is influenced by the distance between the two poly (A) sites. If we invert the two poly (A) sites, the μ_m poly (A) site is used. This poly A region contain DNA sequences which have been shown to block transcription of the δ gene.We suggest that in this DNA construct the second poly (A) site is not used because sequences downstream of the μ_m poly (A) site are not transcribed.

BIO 412

ESTROGEN-DEPENDENT IN VITRO TRANSCRIPTION OF THE XENOPUS VITELLOGENIN B1 PROMOTER

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In Xenopus laevis females, the vitellogenin genes which encode the precursor of the yolk proteins are chronically activated in the liver by estrogen, whereas in males they are silent. With the aim to study the regulation of these genes in their natural context, we have prepared nuclear extracts from Xenopus livers and demonstrate here that they are able to sustain hormone-dependent *in vitro* transcription from the vitellogenin B1 promoter which contains the estrogen responsive element (ERE). Extracts from both estrogen treated males and chronically stimulated females are functional, but only when estrogen is added to the reaction. In addition, liver extracts from uninduced males that have never expressed these genes are incapable of transctivating them, even in the presence of the hormone. Thus, this transcription system mimics the *in vivo* situation and represents the first example of gene regulation by a steroid hormone *in vitro*.

BIO 413

DISTRIBUTION AND LOCALIZATION OF LIPOSOME-ASSOCIATED ^{14}C-DIAZEPAM AFTER INTRAVENOUS INJECTION IN MICE

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After intravenous injection liposomes either degrade rapidly in circulation or they are taken up mainly by the reticuloendothelial system.Precisely, the liver is the major organ responsible for processing and elimination of systemically administered small unilamellar liposomes. Autoradiographic analysis on the ultrastructural level reveals no distinct differences in distribution of the lipophilic drug diazepam associated with either negatively charged or sphingomyelin containing liposomes into the different cell types of the liver, but clearly shows intracellular uptake into parenchymal cells. No redistribution of the released free drug through the blood stream could be observed as shown by the lack of accumulation of radioactivity in the brain.

BIO 414

OMEGON:A SELECTABLE DNA ELEMENT WHICH FACILITATES GENETIC MANIPULATION OF GRAM-NEGATIVE BACTERIA

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We have previously shown that derivatives of the Omega interposon can be used for in vitro mutagenesis of cloned DNA in a wide range of Gram-negative bacteria. Each of these DNA fragments carries a different antibiotic resistance flanked, in inverted orientation, by T4 transcriptional and translational termination signals and by polylinkers. To mutate genes in situ on the chromosome, we have developed a vector called pJFF350 that carries a new interposon, Omegon. This derivative of Omega has the ability to transpose like IS1 and thus allows in vivo insertional mutagenesis. pJFF350 replicates in *E.coli* but not in most other Gram-negatives. The plasmid can be transferred by conjugal mobilization to other Gram-negatives (e.g., *P. putida*). Since the plasmid is not stable in *P. putida*, colonies which have undergone an Omegon transposition event can be identified by selection for the Omegon antibiotic resistance. Once inserted in the chromosome, the Omegon mutation is stable because the transposase gene has been lost. Since Omegon carries the pBR322 replicon, cloning the chromosomal DNA flanking the site of the Omegon insertion is facilitated.

Genetics (GEN)

GEN 415

TRANSPOSITION FREQUENCY OF IS ELEMENTS IN E.COLI

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We present a positve selection procedure for bacterial strains carrying mutations in a specific DNA segment. This DNA segment contains a functional phage lambda cI gene and the lambda p_R promoter which controls expression of the neo gene derived from the kanamycin transposon Tn5.The DNA segment is flanked by symmetrical polylinkers and can therefore be cloned as a cartridge into different replicons.
E.coli and other enterobacterial strains like Enterobacter cloacae and Citrobacter freundii carrying pRAB2 (Cartridge in pUC7) or pRAB1 (Cartridge in a pACYC184 derivative) are Km^s because lambda p_R is repressed by the cI gene product. Spontaneous Km^r mutants were isolated with frequencies of 10^{-7} to 10^{-5}. Rapid plasmid isolation, restriction analysis and plasmid sequencing revealed that 1-30% of the mutations were due to integration of the insertion elements IS1, IS2 or IS5 into the cartridge region of the plasmids.
In cultures of strain 431(pRAB2) incubated for 7days at 30° in stationary or logarithmic growth phase, the frequency of Km^r cells increased about 10-fold in both cases. However, in stationary phase many different insertion mutations were found whereas in logarithmic phase ca. 90% of the Km^r mutations were due to a particular deletion.

GEN 416

RFLPs OF HORSE CLASS II MHC GENES DETECTED BY HUMAN ALPHA AND BETA CHAIN PROBES

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Genomic DNA from 20 horses was digested with up to 6 restriction endonucleases and analysed by Southern blot hybridization to various human class II α- and β-chain cDNA probes. The DQα probe detected the most polymorphism (22 bands), whereas DPβ was apparently monomorphic. The other probes employed detected intermediate polymorphism. A number of interesting correlations of RFLPs with individual MHC allo-antisera were observed.

GEN 417

SOMATIC GENOTOXICITY IN DROSOPHILA: EFFECTS OF 5 CHEMICALS PRODUCING QUALITATIVELY DIFFERENT PRIMARY DAMAGE

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The DNA alkylator streptozotocin, the DNA cross-linker senkirkine, the DNA intercalator mitoxantrone, the base analogue 6-azauracil, and the antimetabolite aminopterine (which produces nucleotide pool imbalances) were studied for their genotoxic effects in the wing spot test of Drosophila. Larvae heterozygous for the recessive wing cell markers mwh and flr, as well as larvae heterozygous for mwh and a multiply inverted chromosome were fed during 48h with each of the 5 compounds. After hatching of the flies, their wings were inspected for the presence of mwh and/or flr mutant spots. The spots are due to (1) mitotic recombination, (2) mutation or (3) deletion in the mwh flr+ / mwh+ flr double heterozygotes. In the inversion heterozygotes, mitotic recombination (1) is suppressed. All compounds induced spots in both types of larvae with a varying proportion of mitotic recombinants depending on the compound used. (Supported by the Swiss National Science Foundation, grant No. 3-657-0.84)

GEN 418

Heterologous gene expression in Saccharomyces cerevisiae with eukaryotic cytochrome P450 genes.

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Cytochrome P450s are hemoproteins involved with NADPH-P450 reductases in the monooxygenation of physiologically important compounds (steroids, fatty acids), of drugs and participate in the metabolism of toxic compounds such as polycyclic hydrocarbons. The versality of these hemoproteins is of interest for specific chemical biotransformations or for the removal of toxic substances from the environment. For this purpose, P450s have to be produced in a convenient recipient. Since the yeast S. cerevisiaehas the ability to biosynthesize hemoproteins and contains a NADPH-P450 reductase, this type of organism was used as a host for the expression of different eukaryotic P450s genes carrying different substrate specificities (Candida tropicalis P450alk, rat P450e, mouse P_1P450, bovine P450$_{17\alpha}$). A maximal expression of these P450s is a prerequisite for the use of yeast cells as biocatalysts. Therefore, the influence of parameters such as choice of yeast expression vector, growth conditions or strain selection on P450 expression have been investigated. A defined P450 type (P450alk) expressed in yeast has been shown to be functional. The utilization of these yeast cells in the hydroxylation of short chain fatty acids has resulted in the accumulation of hydroxylated products in the extracellular culture medium, thus showing the usefullness of whole cells as catalysts. The expression of other P450 genes is in progress.

GEN 419

STRUCTURE AND EXPRESSION OF TWO NEW HOMEOBOX-CONTAINING GENES OF *DROSOPHILA MELANOGASTER*

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Using DNA probes from homeobox-containing genes located outside of the Antennapedia and Bithorax complexes, three homeobox-containing genes were isolated. One clone, W4, is located at 84A and corresponds to the clone S60 that derives from the *zerknüllt (zen)* region of the Antennapedia complex (ANT-C). The other two clones represent two new homeobox-containing genes. Clone W26 maps at 57B on the second chromosome and is expressed during embryogenesis. By Northern blot analysis two transcripts of 2.7 kb and 2.3 kb are detectable. Isolated cDNA clones revealed a complex genomic organisation with at least six exons and different splicing patterns. The homoebox of this gene has an intron and shows the highest degree of homology to the *paired* homeobox, that is 61% at the DNA level and 58% at the protein level. Clone W13 maps at 88A and detects a 2.3 kb RNA during embryogenesis. Also for this gene cDNA clones have been isolated and characterized. The structure of both genes and their expression as studied by *in situ* hybridization will be presented.

GEN 420

REGULATION OF HLA-CLASS II GENES: IDENTIFICATION OF A REGULATORY PROMOTER BINDING PROTEIN MISSING IN CLASS II-DEFICIENT CONGENITAL IMMUNODEFICIENCY (SCID)

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HLA-class II gene expression has been studied in two systems; induced expression by IFN-γ in fibroblasts and constitutive expression in B cells. Induction by IFN-γ is transcriptional, occurs after a 6-9 hour lag phase and is mediated by de novo synthesis of a class II inducer protein. To study constitutive expression we analyzed DRα promoter binding proteins in normal B cells and in B cells from class II deficient severe combined immunodeficiency (SCID) patients. This allowed us to identify a class II X box binding protein (RF-X) that is present in normal B cells but deficient in SCID cells. The SCID defect thus lies in RF-X, a regulatory protein required for class II gene transcription. The absence of RF-X correlates with the absence of 2 DNAaseI hypersensitive sites normally found in the DRα promoter. These 2 sites are found in normal B cells and in both uninduced and induced fibroblasts. RF-X does therefore not appear to be B cell specific.

GEN 421

THE HEPATOCYTE-SPECIFIC PROMOTER ELEMENT HP1 IS RECOGNIZED BY TRANSCRIPTION FACTORS AND IS COMMON TO SEVERAL GENES SPECIFICALLY EXPRESSED IN LIVER CELLS

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By transfecting various Xenopus albumin-CAT fusion genes into the mouse hepatoma cell line BWIJ, we have identified a 13bp hepatocyte-specific promoter element, HP1. This cis-acting element is recognized by nuclear factors specific for hepatic cells as shown by band-shift assays with extracts from various cell lines. In vitro transcription experiments in a rat liver nuclear extract demonstrate that HP1 acts as a positive transcriptional element and that the increased transcriptional activity caused by HP1 can be specifically inhibited by the addition of an oligonucleotide containing HP1. Promoter sequence alignement reveals that sequences homologous to HP1 are also present in the albumin and alpha-fetoprotein genes of other vertebrates and even in other genes specifically expressed in the liver, e.g. human α_1-antitrypsin, human transferrin, rat fibrinogen and the Xenopus vitellogenin A2 gene. Direct evidence that a common promoter element is present in the Xenopus albumin and the human α_1-antitrypsin gene, is obtained by our observation that the HP1 oligonucleotide inhibits the in vitro transcription of a human α_1-antitrypsin gene construct.

GEN 422

MBP GENE DUPLICATION IN *MLD* MICE DOES NOT AFFECT TRANSCRIPTION INITIATION

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Myelin deficiency (*mld*) is an autosomal recessive mutation in mice characterized by hypomyelination of the CNS. We have shown previously that the mutation causes low levels of normal myelin basic protein (MBP) and low steady state levels of MBP specific mRNA indicating that the mutation is not in the coding region of the MBP gene. Therefore, the *mld* mutation could affect either transcription or the stability of the messengers. Southern blot analysis of *mld* genomic DNA using a cDNA probe did not show any detectable difference in the introns, but revealed a gene duplication. The same duplication was found in different strains of mice and genetic experiments (Akowitz, A. A. et al., *Genetics* 116: 447, 1987) showed cosegregation of the duplicated gene with the mutation itself. Genomic DNA Southern analysis using 5' end of the MBP gene as a probe confirmed the duplication at the 5' end, and preservation of at least 3 kb upstream from Exon I. *In vitro*, transcription initiation was not decreased in *mld* mice, indicating that the duplication does not affect gene transcription and that the *mld* mutation probably alters the stability of specific mRNAs.

GEN 423

STRUCTURAL AND BEHAVIORAL CHANGES IN LOZENGE^3, A DROSOPHILA MUTANT LACKING ANTENNAL BASICONIC SENSILLA

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The mutant lozenge^3 (lz^3) of D. melanogaster, which is known for eye, tarsal and gonadal defects, also controls the antennal sensillum pattern: Coeloconic sensilla are increased in number, whereas basiconic sensilla are completely lacking, leading overall to a loss of antennal afferents. In the antennal commissure, these afferents are even more reduced. This suggests that lz^3 is also involved in regulating the proportion of antennal fibers which extend into contralateral brain regions. Another highly specific change in the brain of lz^3 is the lack of glomerulus V, which is a major target of basiconic afferents. Using mosaic flies exhibiting a lz^3 antenna it can be shown that the lack of V is causally related to the loss of basiconic sensilla. We conclude that glomerulus V is an association center of basiconic input. Under identical assay conditions, courtship vigor and copulation latency of lz^3 males toward virgin or mated wildtype females is similar as for wildtype males. This suggests that basiconic sensilla are not involved in the perception of female attraction or inhibition pheromones.

GEN 424

CHROMOSOMAL LOCALIZATION OF TWO HUMAN Mx GENES

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In human cells, type I interferon induces the synthesis of two distinct, Mx-related mRNAs. Using cDNA probes with specificity for one or the other of these mRNAs, we have determined the chromosomal localization of the corresponding genes. Southern blot analyses of genomic DNA samples isolated from 14 independent mouse- human cell hybrids indicate that both human Mx- related genes are located on chromosome 21. Interestingly, the mouse Mx gene is located on the distal part of mouse chromosome 16 in a region of genetic homology to human chromosome 21. This is a further example of homologous gene clustering in different species.

GEN 425

CLONING OF cDNA AND LOCALIZATION ON HUMAN CHROMOSOME 21 OF THE INTERFERON-INDUCED GENE CODING FOR THE HUMAN EQUIVALENT OF THE MOUSE PROTEIN Mx.

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The murine Mx protein is induced by type I interferon (IFN) and it mediates specific resistance to influenza viruses. Recently we have purified to homogeneity the human equivalent of the murine Mx protein (Horisberger & Hochkeppel, J. Interf. Res. 7, 331-343, 1987). A partial sequence at the NH_2-terminus has allowed us to screen a cDNA bank constructed from type I IFN-induced human diploid fibroblasts. cDNA clones were characterized by hybrid selection, and sequencing revealed extensive amino acid homologies with the murine Mx protein. The gene was mapped to chromosome 21 (encoding also the IFN receptor and is involved in Down's syndrome and Alzheimer's disease) by human x rodent somatic-cell hybridization, and by gene dosage using chromosome 21 trisomic cells.