Biol. Chem. Hoppe-Seyler
Vol. 370, pp. 869–978, September 1989

Herbsttagung der Gesellschaft für Biologische Chemie

13.–15. September 1989 in Osnabrück

Autorenreferate der angemeldeten Vorträge und Poster

(Die Referate sind nach der alphabetischen Reihenfolge der *Erstautoren* geordnet)

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Localization of early light-inducible protein (ELIP) in the thylakoid membranes of pea

ELIPs are nuclear coded chloroplast proteins of higher plants. The pea protein has a precursor of 24 kD and a product of 17 kD. The expression of its mRNA culminates at 2-4 hrs after starting the illumination of etiolated peas that of the protein at 8 hrs as detected by decoration with the antibody. ELIP is also induced in green plants which were transfered to darkness for 36 hrs and then exposed to light for 4-6 hrs.

The localization of ELIP in the thylakoid membrane was investigated by "nearest-neighbour analysis" using two cleavable crosslinking agents DSP[1] and SAND[2]. The crosslinked products were separated by a diagonal gel electrophoresis. Analysis of the off-diagonal spots pattern produced by cleavage of crosslinkers and second dimensional electrophoresis was made on the basis of a coomassie blue or silver staining and labeling of ELIP with ^{35}S-methionine. Using SAND we identified ELIP in a 41-51 kDa crosslinked product while with DSP three products of 21-23 kDa, 42-50 kDa and 70-80 kDa were found.

After fractionation of thylakoid membranes into the various protein complexes done after post-translational transport and crosslinking with SAND we observed the 17 kDa protein mostly in the fraction enriched in photosystem II particles.

1) Dithiobis(succinimidylpropionate)
2) Sulfosuccinimidyl 2-(m-azido-o-nitrobenzamido)-ethyl-1,3'-dithiopropionate

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Neuroleptic receptors in nematodes

Acetylcholine, serotonin and dopamine have been implicated as putative neurotransmitters in parasitic worms [1,2]. By treatment of the filarial nematode Brugia pahangi with various serotonin and dopamine antagonists a paralysing effect was shown, but it remains for elucidation that this effect results from interaction with serotonin and dopamine receptors. Binding assays with membrane preparations of the model nematode Ascaris suum revealed binding sites for the neuroleptics haloperidol, spiperone and mianserin, which are known to bind with high specificities to mammalian D_2, D_2/S_2 and S_2/H_1 receptors, respectively. Saturation experiments, however, showed K_D values of approximately 2µM, which are in contrast to values in the pM to nM range reported from mammalian receptors. Various compounds - known from vertebrates to compete specifically with binding of haloperidol, spiperone and mianserin, respectively - demonstrated similar potencies in reducing binding of these neuroleptics in case of Ascaris, results which provide evidence for a nematode receptor of unique properties.

In addition to this neuroleptic binding site preliminary results indicate the occurrence of

an acetylcholine receptor with specificity for α-bungarotoxin. This ligand could not be displaced by any of the three neuroleptics and for this reason was considered to label a distinct binding site.

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Protein Import in Chloroplasts: Description of the Chloroplast Envelope Localized Translocation Process

Binding of precursor proteins to the outer chloroplast envelope occurs at distinct sites in the membran and not random, as shown by electron microscopic studies. Using chimeric proteins we examined whether precursor proteins span both the outer and the inner chloroplast envelope membrane simultaneously. The data of the transport experiment suggest that, as shown for mitochondrial protein translocation, translocation intermediates exist also for chloroplasts. We compare import data obtained from transport experiments using precursor proteins synthesized by in vitro transcription-translation systems and a precursor protein overproduced and purified from E.coli.

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A Computer-Generated Model of a Glucocorticoid-Carrier in the Rat Liver Plasma Membrane

The classical concept of the action mechanism of steroid hormones that assigns a functional role exclusively to their respective intracellular receptors is being increasingly confronted with a body of findings suggesting that plasma membrane components participate in the uptake process of steroids by target cells. We had several times reported on a presumptive carrier with high affinity (mean Kd=7.2 nM at 296 K) for corticosterone inserted in the rat liver plasma membrane (PM) that may functionally be involved in the transport of glucocorticoids (GC) into the hepatocyte [1-6]. We now present a 3-dimensional model of this carrier generated from structural and experimental binding data of a set of ligands by a newly developed automated receptor modeling method (ARM) [7]. The model provides insight into the interactions between the ligands and the carrier and thus leads to an understanding of the different affinities of various steroid hormones. E.g., the low energy conformations of the free molecules cortisol (F) and prednisolone differ considerably by the orientation of the 21-OH group. The interaction of the OH-group with the carrier is very significant and therefore leads to a more favorable overall interaction of prednisolone. An explanation for the high affinity of F in contrast to cortisone can also be given: The positively polarized hydrogen of the 11ß-OH group in F gives rise to a weak but attractive interaction with the carrier in that region, whereas the 11-carbonyl oxygen with a negative partial charge in cortisone leads to a weak repulsion. The validity of the carrier model has been demonstrated by correct prediction of affinities of several steroids which were not part of the set of ligands used to generate the model. The Kd-value of ethinodiol, which has not been investigated experimentally yet, is predicted to be 3 nM.

These recent findings may corroborate the assumption that a specific plasma membrane-inserted carrier is an essential prerequisite for glucocorticoids to exert their bioactivity.

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Proton Conductance by CF_O, the Channel Portion of the Chloroplast ATP Synthase

The chloroplast ATP synthase, CF_OCF_1, synthesizes ATP in the CF_1 portion driven by proton flow which enters the enzyme through the channel portion CF_O. Kinetics of proton transport across CF_1-depleted thylakoid membranes have been measured spectrophoto-

metrically under flashing light by electrochromic and pH-indicating absorption changes. The time-averaged single channel conductance of CF_0 was determined, appr. 1pS equivalent to $2 \cdot 10^5$ $H^+/(CF_0 \cdot s)$ at 30mV driving force. Comparison of the kinetics of the charge transport with the kinetics of proton transport via CF_0 (measured by pH-indicating dyes) has revealed that CF_0 is proton specific even at pH8 and against a background of 300mM monovalent cations (selectivity greater than 10^7). Between pH5.6 and pH8 proton conduction of CF_0 was independent of the medium pH. In D_2O, the conductance of CF_0 was decreased by a constant factor of 1.7 over the whole accessible pH/pD range. The activation energy of proton translocation is 42kJ/mol in H_2O and 47kJ/mol in D_2O. Addition of glycerol lowered the measured conductance of CF_0 and diminished the effect of isotopic substitution. CF_0 is kinetically competent to serve as a low impedance access to the protonic coupling site in the integral ATP Synthase, CF_0CF_1. The activation energy of proton conduction by CF_0 is intermediate between the ones for diffusion and chemical reactions. The full conduction cycle comprises at least two reactions in series, where a non-protonic one (diffusion controlled) becomes rate limiting in glycerol.

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Conductance and Dimerization Behaviour of Gramicidin in Photosynthetic Membranes

It is generally accepted that Gramicidin acts as an ion pore in biomembranes by head-to-head dimerization of two monomer helices. In seeming contradiction to the quadratic concentration dependence in other membranes (i.e. from sheep red cells, Tosteson *et al.*, 1968) the conductivity in thylakoid membranes increases linearly as function of the concentration of added gramicidin (100 pM to 2 nM; Junge, 1968). Did gramicidin then act as a monomer or was it always dimerized?
With flash spectrophotometry we determined the membrane conductivity of thylakoids from *Spinacia oleracea* and *Pisum sativum* as well as of chromatophore membranes (*Rhodopseudomonas sphaeroides*) in the range of 1 pM to 10 μM gramicidin in suspensions with about 150 μg protein/ml. Significant deviations from the linear dependence were observed at very low gramicidin concentration (1 pM to 100 pM). This showed that the dimerization model was valid also for photosynthetic membranes. Linear dependence in the range from 100 pM to 100 nM gramicidin, as also found in earlier studies, is due to a very large dimerization constant ($\geq 10^{14}$ cm^2M^{-1} in photosynthetic membranes, compared to 10^{11} cm^2M^{-1} in glycerolester membranes and 10^{13} cm^2M^{-1} in phosphatidyl choline membranes (Veatch *et al.*, 1975)).
There are two possible reasons for the large apparent dimerization constant in photosynthetic membranes. Lower membrane thickness and, due to the high protein content, less membrane area where gramicidin is free to diffuse. The higher dimerization constant justified our previous calibration of the conductance of CF_0, the proton channel of the chloroplast ATP synthase, against the known unit conductance of gramicidin (Lill *et al.*, 1987).

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Spectroscopic and catalytic properties of terminal oxidases from *Sulfolobus acidocaldarius*

1. Membranes of the thermoacidophilic archaebacterium *Sulfolobus acidocaldarius*, grown heterotrophically at 80°C under aerobic conditions, contain various b- and a-type cytochromes, whereas c-type cytochromes are absent. These hemoproteins can be visualized by difference spectroscopy of whole cells, exhibiting absorption maxima at 440, 562, 586 and 605 nm.
2. Spectra of the isolated plasma membrane show a higher resolution in the α- and ß-region exhibiting maxima at 531 and 537,5 nm (ß-bands), as well as 559 and 565,5 nm (α-bands). The latter absorptions are attributed to b-type cytochromes, while the maximum at 605 nm results from cytochrome aa_3 which could be partially purified (1). The additional absorption at 585,5 nm though being the most prominent spectral contribution could not be asigned unequivocally so far. It appears possible that it is a composite spectrum of several components, a-type, b-type cytochromes and eventually free heme-a.
3. In purified aa_3 preparations (1) besides the typical 604 nm absorption peak variable amounts of a 587 nm band were observed, clearly resulting from heme-a. An improved isolation procedure using hydroxyapatite chromatography allows the preparation of material containing only the 604 nm α-band. A fraction eluting at higher salt concentration exhibits both, 587 and 604 bands. However, in both cases only one single polypeptide (38-40 kDa) was identified on SDS-gels as the major protein constituent. It appears likely that the 587 nm band is due to partial denaturation of aa_3. Moreover, as yet no proteinfraction could be isolated carrying only the 587 nm heme component. Nevertheless, in intact cells or freshly prepared membranes the respective absorption bands at 586 (585,5)nm have to be associated with native protein components; thus, its functional attribution remains enigmatic.
4. One b-type cytochrome functionally is considered to be cytochrome-o as concluded from CO-difference spectra and the finding (2) of a high potential heme-b (+400 mV), appropriate for a terminal oxidase.
5. The detergent solubilized and purified aa_3 preparation (1) has negligible capacity to oxidize cytochrome-c. It is capable to oxidize reduced TMPD (K_M100μM); the reaction is 100% cyanide sensitive. The TMPD/O_2 stoichiometry is 4, thus indicating the reduction of O_2 to water. Interestingly, the highest turnover was obtained with caldariella quinone. The quinone oxidizing activity is purified to the same extent as the heme-a content. Therefore, this enzyme containing 2 heme-a centers and 2-3 coppers may represent a novel type of aa_3-terminal oxidase.

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J. Arendes and M. Oed

DNA sequences from the small eukaryote Nanochlorum eucaryotum with ARS activity in yeast

High frequency transformation of Saccharomyces cerevisiae was used as a functional assay to

isolate autonomous replicating sequences (ARSs) from the small unicellular marine alga Nanochlorum eucaryotum. Because the presence of sporopollenin in the cell wall of N. eucaryotum prohibited the isolation of intact subcellular organelles (1), chloroplast DNA was enriched by repeated CsCl density centrifugation. HindIII-fragments of the ctDNA were cloned into a vector which contains the yeast leu2 gene and is not capable to replicate autonomously. DNA fragments were isolated on the basis of their ability to confer replication on chimeric plasmids in yeast

In addition to the previously described 2.2 kb ARS element (2), we isolated a 1.9 kb fragment with ARS activity. This element was subcloned to further define the unique region of ARS activity. The activity was shown to be in a 1.15 kb fragment which has an A+T content of about 75%. DNA sequence analysis revealed that the element contains the yeast consensus sequence and enhancer-like sequences in the flanking regions. The DNA sequence shows also strong homologies to mitochondrial origins as well as cytochrome genes. Wether these similarities in sequences are derived from a putative sequence transfer between organelles or are due to impurities in the ctDNA remains to be analyzed.

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Rat liver fat storing cells are stimulated by autocrine mechanisms via transforming growth factors

Activated Kupffer cells (1,2) and platelets (3) stimulate via paracrine mechanisms rat liver fat storing cell (FSC) proliferation/transformation and the synthesis of extracellular matrix elements. Several polypeptide growth factors including transforming growth factors seem to be involved in this process leading to enhanced fibrogenesis. In the present study we demonstrate an autocrine stimulation of transformed FSC (myofibroblast like cells = MFB) via Transforming growth factors (TGFa,TGFß1).

Methods: Rat liver FSC were isolated by the collagenase/pronase method and purified by a single step Nycodenz density gradient. FSC in primary culture were stimulated by conditioned medium from MFB (MFBCM) or during coculture experiments with MFB. To examine the presence of the latent form of TGFß the MFBCM was acidified by HCl (30min. pH 2) and the stimulatory effects were compared to the unacidified MFBCM. Cell proliferation, sulphated proteoglycans and the glycosaminoglycan hyaluronic acid, respectively, were measured. In radioreceptor assays using [^{125}I]TGFß or [^{125}I]EGF the presence and concentration of TGFß and the EGF analogous TGFa in MFBCM was determined.

Results: MFBCM stimulated in a dose dependent manner FSC proliferation/transformation and proteoglycan synthesis of FSC in primary culture grown in DMEM with 0.5% fetal calf serum. Similar results were obtained in coculture (FSC prim. culture in coculture with MFB like cells) experiments. Acidification of the MFBCM enhanced the proteoglycan synthesis stimulating activity (from 1.2fold to 2.1fold) but inhibited FSC proliferation. Radioreceptor experiments showed that MFBCM contain EGF/TGFa (10-20 ng/ml) and acidified MFBCM contain active TGFß (1-5 ng/ml). These results indicate that TGFa and TGFß (primary inactive form) is released by myofibroblast like cells. Beside paracrine stimulation of FSC this study shows for the first time an autocrine stimulation of transformed FSC (MFB) via TGFa (proliferation) and TGFß (transformation and proteoglycan synthesis). This mechanism might be important as selfperpetuation of the fibrogenic process.

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Characterization of the Autoantigen La, an RNA polymerase III transcription/termination factor, as an ATPas/dATPase with melting properties: Analysis of its nucleocytoplasmic shuttling.

Sera of patients' with autoimmune diseases often contain autoantibodies recognizing the so called La antigen. This protein is known to associate with so far known all RNA polymerase III transcripts, and in addition to some RNA polymerase II transcripts. Its binding site on RNAs is a 3'terminal oligo(U) sequence, which is usually added during transcription termination of RNA polymerase III reaction. In the case of the few RNA polymerase II transcripts being able to directly associate with the La protein their oligo(U) sequence is posttranscriptionally added. Analyzing the intracellular localization of La protein we found that it colocalizes with clusters of interchromatin granules (ICGs) and perichromatin regions in the nucleus and with the rER in the cytoplasm as analyzed at the level of immunofluorescence- and electronmicroscopy (1-5). ICGs are assumed as storage regions for unused nuclear material. The ICGs were colocalized using mabs directed against ICGs, but also with mabs to U snRNPs. During these studies we observed a nucleocytoplasmic shuttling of La protein depending on the activity of RNA polymerase II (3,5). This shuttling presumably represents a cotransport of complexes formed between

certain La RNPs (4.5 S RNPs, Ro RNPs) by hybrization of their 3'terminal oligo(U)-tail with the poly(A) sequence of mRNAs (3-5). Such complexes are translationally inactive. After infection of CV-1 cells with herpes simplex virus type 1 the observed shuttling was strongly altered (3,5,6). Recently La protein was characterized as a transcription termination factor of RNA polymerase III. Consequently it remains unclear (i) how can La protein terminate transcription of RNA polymerase III and (ii) why shuttles a transcription termination factor of RNA polymerase III together with RNA polymerase II transcripts between the nucleus and the cytoplasm. To solve these problems we investigated La protein for enzyme activty. We found that homogenously purified La protein and also a newly prepared recombinant La protein becomes an ATPase/dATPase in the presence of DNA-RNA- or RNA-RNA hybrids (7). Moreover, immunoabsorbed patient anti-La antibodies and also a carefully characterized novel anti-La mab was able to inhibit enzyme activity of La protein (7). In dependence on this enzyme activity La protein was able to melt a hybrid formed between oligo(U) and poly(A) in vitro (7). Consequently it seems likely that La protein has a common function during transcription termination and shuttling of La protein, namely the melting of DNA-RNA- or RNA-RNA helices (7).

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T. Bakker-Grunwald and U. Löhden-Bendinger

Protein Glycosylation in *Entamoeba histolytica*: Evolutionary Implications

In higher eukaryotic cells, endoplasmic reticulum (ER) and Golgi are the sites of protein glycosylation and sugar side-chain trimming, respectively. *E. histolytica* has been classified as an archaezoon[1]; although eukaryotic, it has a very simple morphology, lacking a Golgi and a well-developed ER. Wih this in mind we investigated the incorporation of ^{3}H-mannose into protein in these amoebas.
After a 20-min time lag, ^{3}H-mannose (extracellular concentration, 1 μM) was incorporated at a constant rate (0.4 nmol.h^{-1}.g^{-1} wet wt) into trichloroacetic acid-precipitable material; as in higher eukaryotes, this incorporation was tunicamycin-sensitive (halfmaximal inhibition at 10 μM). By a recently-developed method based on magnetic separation[2] we established that virtually no ^{3}H-mannose was incorporated into pinocytic vesicles.
This latter result confirms on the functional level that *E. histolytica* possesses an ER-like compartment. It suggests that relegation of protein glycosylation to specialized subcellular structures may have occurred very early in evolution. As a practical aspect, no marker has been available yet for the ER in *E. histolytica*; based on our results, we propose that ^{3}H-mannose incorporation may be used as such.

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The biochemistry of the sex inducer of Volvox carteri

The green flagellate Volvox carteri turns from vegetative to sexual reproduction by an external signal excreted on disintegration of mature sperm packets . The signal compound has been purified to homogeneity from sperm packet supernatants. By reversed phase HPLC the glycoprotein is separated into several isoinducers which have the same protein core as demonstrated by chemical deglycosylation. The amino acid sequence of the inducer, including its long leader sequence, has been determined via a cDNA library constructed in the vector lambda gt 10. It contains six possible N-glycosylation sites, three of them (in the centre of the molecule) are substituted by oligosaccharides. These were isolated enzymatically with glycopeptidase F (EC 3.2.2.18) and by hydrazinolysis. After fluorescence labelling with 2-aminopyridine five main components were separated and partially characterized by exoglycosidase digestion and monsaccharide analysis. The oligosaccharides are of complex type and composed of N-acetylglucosamine, mannose and xylose. Part of the possible O-glycosylation sites are set by chains composed of xylose, arabinose and galactose. The carbohydrate moiety is essential for biological activity; only the two most highly glycosylated

isoforms induce. The biosynthesis of the inducer during sperm packet maturation was investigated by means of specific antibodies and immunoblot analysis. Protein biosynthesis and glycosylation occurs in mature sperms just a few hours before disintegration. The inducer was coupled with biotin and a photoreactive reagent in order to check for binding sites on spheroidal membranes by the avidin-peroxidase blot technique. After SDS-PAGE a band of 240 kDa was detected, whose labelling is displaced by excess unlabeled inducer.

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Regenwurm (Lumbricus terrestris) Erythrocruorin als Eichsubstanz in der Gelchromatographie

Die Gelchromatographie stellt wegen ihrer Einfachheit eine verbreitete Methode dar, Molekulargewichte (MG) der Makromoleküle sowie deren Verteilung zu bestimmen. Heute stehen insbesondere für den Bereich hoher Molekulargewichte gute Trenngele zur Verfügung. Voraussetzung für solche Bestimmungen sind jedoch Eichsubstanzen, deren Dichte und Form den zu analysierenden Molekülen möglichst ähnlich sind. Wir standen vor dem Problem, künstlich hergestellte polymere Hämoglobine analysieren zu müssen. Während im kleinen Molekulargewichtsbereich geeignete Eichproteine verfügbar sind, fehlen solche für den hohen Bereich. Daher untersuchten wir das Regenwurm-Hämoglobin auf seine Brauchbarkeit als Eichsubstanz der Gelchromatographie.

Gewinnung der Substanz: Durchschneiden des Regenwurms in Höhe des 7. Segments, Aufnehmen der am Körperstück austretenden roten Flüssigkeit mit einer Kapillare. Man gewinnt etwa 50 µl einer 6%igen Hämoglobinlösung (Cyan-Hämiglobin-Methode). Die Menge reicht für mehrere Chromatogramme.

Gelchromatographie: Säule 1 cm innerer Durchmesser, 80 cm Länge, Füllung mit Sephacryl S 400 HR (Pharmacia, Freiburg, BR Deutschland), Elutionsmittel (in mmol/l): NaCl 125; KCl 4,5; $NaHCO_3$ 20; pH = 8,6; Temp.: 22 °C; Fluß: 5,3 ml/h; optische Detektion bei 425 nm mit Durchflußküvette 1 cm Schichtdicke; Ausschlußvolumen (Vo) mit Dextran-Blau (Pharmacia) und gesamtes Volumen (Vt) mit Glutathion (Merck, Darmstadt, BR Deutschland) gemessen. Eichproteine: Ribonuklease A 13700, Rinder-Albumin 67000 und Thyroglobulin 669000 g/mol (alle von Pharmacia). Volumenfaktor VF = (Ve-Vo)/(Vt-Vo), Ve = Elutionsvolumen der betreffenden Substanz.

Chromatogramm des Regenwurm-Hämoglobins zeigt ein Hauptmaximum ($\overline{VF}$ = 0,25) und Nebenmaximum zu kleinen Molekulargewichten (VF = 0,56), letzteres hat etwa 1/10 der Höhe des Hauptmaximums. Der Zerfall des Moleküls unter bestimmten Bedingungen in (12) Untereinheiten ist bekannt[1]. Die VF-logMG-Werte der käuflichen Eichproteine liegen genau auf einer Geraden. Mit dieser Eichgeraden und den VF-Werten ermittelt man für das Hauptmaximum ein Molekulargewicht von $2,9 \times 10^6$ g/mol und für das Neben-Maximum 275000 g/mol. Das Molekulargewicht des Hauptmaximums liegt nahe bei bekannten Literaturwerten[1], gemessen mit Hilfe der Ultrazentrifugation und Lichtstreuung. Der Wert beträgt $3,06 \times 10^6$ g/mol, ihn schlagen wir als Eichwert des Hauptmaximums vor. Durch die Existenz des Nebenmaximums bietet das Hämoglobin des Regenwurms zugleich auch noch einen Eichpunkt im unteren Molekulargewichtsbereich.

Mit der Verwendung des Regenwurm-Hämoglobins schließt sich eine Lücke für Eichproteine der Gelchromatographie im hohen Molekulargewichtsbereich.

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Ferro-magnetic isolation of oocyte endosomes

We use the oocytes of Xenopus laevis as a model system for studying the transport pathway and lysosomal processing of the yolk-precursor vitellogenin. A novel method has been designed to employ ferric particles, conjugated to the vitellogenin, as a marker for pinocytic compartments: 1. in order to follow the route of incorporated vitellogenin inside the intact oocyte from the oolemma into the yolk organelles via coated pits, coated vesicles, fused tubular endoplasmic reticulum, and multivesicular endosomes which contain crystalline lipoproteid yolk; 2. to achieve magnetic sorting of the different functional compartments involved in lipoprotein uptake. After pulse-chase loading of these endocytic compartments with 10nm ferric particles, the oocytes are homogenized and subcellularly fractionated. Isolation of the ferro-endosomes is performed with a "free-flow" magnetic chamber and subsequent ultra-centrifugation. The endocytic compartments have been subjected to freeze-fracture and ultrathin-section electron microscopy and to SDS-gel electrophoresis.

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G.Bechmann, A. Zweck and H.Weiss

Regulation of Mitochondrial Ubiquinol-Cytochrome c Oxidoreductase by the Membrane Potential

The following results gives rise to the assumption that the reaction pathway in cytochrome reductase (E.C.1.10.2.2.) isolated of Neurospora crassa is changed by the membrane potential:

1. The $H^+/2e^-$-stoichiometry determined as well by comparing H^+-translocation with cytochrome c reduction as by analysing the flow-force relation of the electron transfer reaction decreases from 2 to 1 when the membrane potential is increased from 0 to 90 mV [1].
2. Under non-potential conditions nor one antimycin per dimeric enzyme inhibits $decQH_2$-cytochrome c reduction neither one myxothiazol inhibits the oxidant induced pre-steady-state cytochrome b reduction by DQH_2. In the enzyme exposed to a membrane potential both reactions are 50% inhibited by one molecule of these inhibitors per dimer.
3. While the quinol-quinone transhydrogenation-activity of cytochrome reductase [2] is inhibited by a membrane potential of 90 mV, the QH_2-cytochrome c reduction activity remains almost uneffected.

We suggest a Q-cycle mechanism, in which electron transfer occurs between the two cytochromes b_H of the cytochrome reductase dimer. This mechanism is changed by the membrane potential in the way that the two monomeric units of dimeric cytochrome reductase work independently to each other. The lower $H^+/2e^-$-stoichiometry of the potential induced pathway can be explained by assuming that in the monomer a semiquinone is translocated from centre o to centre i according to the modell suggested by Wikström and Krab [3].

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A. Bertrams, K. Ziegler

Uptake of Renin Inhibitors into Liver Cells: Evidence for an Active Transport Related to the Sinusoidal Bile Acid Transport

Linear peptides with renin inhibitory activity have a short plasma half-live because of a rapid extraction from portal blood by the liver. Several other xenobiotics with peptide structure (e.g. phalloidin, somatostatins) are eliminated in this manner by the multispecific bile acid transporter. To elucidate the relationship between this carrier and the elimination of renin inhibitors we investigated the effect of such linear peptides on the uptake of natural and foreign substrates of the bile acid transporter into isolated hepatocytes. Renin inhibitor EMD 51921 shows competitive inhibition in all cases, while some other tested compounds show differences between the kinetics of inhibition of cholate- and taurocholate uptake. AS-30D ascites hepatoma cells, which are not able to transport substrates of the bile acid transporter, do also not take up EMD 51921. EMD 51921 is taken up by liver cells in a saturable- and a non-saturable manner. The K_m of the saturable transport is 2 µM, V_{max} is 160 pmol/mg x min. The permeability coefficient is 8,1 x 10^6 cm/sec. The activation energy of 60 kJ/mol and a decrease of transport under anaerobic conditions and in the presence of metabolic inhibitors provide strong evidence for an active transport. Sodium dependence of the transport can be concluded from an inhibitory effect of sodium-free buffers and sodium-selective ionophores. Results after substitution of Cl^- by several anions show the dependence on membrane potential.

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J.Bichler and R.G. Herrmann

Light-dependent and tissue-specific expression of several nuclear genes from spinach

Various segments of 5' upstream regulatory regions of three nuclear genes coding for the chloroplast proteins plastocyanin (PC), subunit delta of the thylakoid ATP-synthase (atpD) and one member of the rbcS multigene family from spinach (var. Monatol) were fused to the GUS cassette and the promotor-GUS constructs introduced into **N. tabacum, var. Samsun NN** via the **A. tumefaciens** vector pBIN19. The transcription of these genes is photocontrolled. Positive light-stimulated regions, a region controlling constitutive, light-independent expression and elements with 'silencer-like' activity in the dark have been defined.

All 5' sequences show a discrete organ, tissue and cell specificity. Highest activity of the three promotors is correlated with the presence of chloroplasts, but the 5' regions of atpD and PC also show clear expression in the phloem regions of leaves, leaf and floral stems, and in the vascular areas of anthers. No histochemical staining has been detected in roots. In floral stems nucleotides -590/+62 in the atpD promotor direct GUS-expression in the inner phloem region and the -1137/-590 promotor fragment is responsible for activity in outer phloem areas. In addition, the CaMV-35S-GUS fusion also reveals tissue-specific expression, low activity in the vascular cylinder of roots, intense staining in the phloem regions of leaf and floral stems and in the vascular tissue of anthers. Constitutive, overall expression is seen in ovaries (1).

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S. Bickel-Sandkötter and P. Strümper

Characterization of Nucleotide Binding Sites on Isolated Chloroplast ATPase by Modification with 7-Chloro-4-nitrobenzofurazan (NBD-Cl)

Addition of NBD-Cl to isolated CF_1 at pH 7.5 is paralleled by formation of a new chromophore with an absorption maximum at 385 nm, which belongs to a tyrosine-NBD-adduct (1). Modification as a function of time is biphasic, showing an initial rapid and a slow kinetic component. Analysis of the rapid and the slow phase in terms of two superimposed exponential processes, yields the first order rate constants of 0.220 min^{-1} (rapid phase, 35% of bound NBD) and 0.066 min^{-1} (slow phase, 65%).The reaction was completed in about 30 minutes. After this time, 1.6 NBD/CF_1 could be found, indicating two possible reaction sites per CF_1.

Addition of excess ADP resulted in the reduction of bound NBD-Cl to a maximum of 1 NBD/CF_1, an monophasic absorption increase with time, and a rate constant of comparable order to that of the initial rapid phase (s. above).

Addition of ATP in presence of Mg^{2+} did not reduce the maximum value of bound NBD, but resulted in a monophasic increase in absorption comparable to the slow phase of the initial curve.

Without nucleotides and in presence of excess ADP, reaction of only a single NBD-Cl molecule per CF_1 is necessary for complete inactivation of ATPase activity. In presence of MgATP, however, complete inactivation is obtained when 1.6-2 NBD molecules are bound per CF_1.

The intramolecular migration of NBD from Tyrosine to Lysine has been observed for one specific Lysine (K_{162}, on ß-subunit of Bovine MF_1) (2). Such transfer can be observed in CF_1 too. The formation of N-lys-NBD is one order of magnitude slower than formation of O-tyr-NBD. Without preformation of O-tyr-NBD at pH 7.5, the absorption change (475 nm for N-lys-NBD) shows a lag-phase of about 4 minutes, which disappears after preincubation of the enzyme with NBD-Cl, indicating that the lag-phase is due to formation of O-tyr-NBD as an obligatory intermediate.

As only one Tyrosine (Y_{311}, Bovine MF_1) has been found labeled with (^{14}C)NBD on MF_1 (3), and at pH 9 up to three NBD bind to CF_1, the results can be interpretated in the following way:

Assuming three identical tyrosines which can react with NBD, each of them on one of the three ß-subunits, one of these tyrosines must be more exposed than the others (the one close to the catalytically active site) and, therefore, be able to react faster. These experiments, however, can not exclude the possibility of two different tyrosines on one ß- subunit. Experiments to determine the modified tyrosine(s) on chloroplast F_1 are in progress.

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Investigation of Reaction Centers of Rhodopseudomonas viridis

Several photosynthetic systems have been investigated by Mössbauer spectroscopy, because iron is used at many steps of the photochemical and enzymatic processes. Here we discuss first experiments on crystals of ^{57}Fe enriched reaction centers (RC) of the photosynthetic membranes from Rhodopseudomonas viridis[1], where the structure is known from X-ray crystallography[2]. The crystals contain only the non-heme iron of the RC and the tetra-heme cytochrome c. Interpretation of the measured spectra shows that the Mössbauer data can be fitted only assuming three different iron species: The cytochrome irons are in the low spin ferric state. The non-heme iron is ferrous high spin in some molecules and ferrous low spin in others (or ferric high spin). In agreement with the X-ray data the ratio of the non-heme iron to cytochrome iron is 1 to 4.

The reduction of the crystals with sodium ascorbate produces a new component in the Mössbauer

spectra with the hyperfine parameters near to those of other reduced cytochromes[3], identifying a ferrous low spin complex. As it is known ascorbate reduces only the two high-potential cytochromes[4]. Under conditions used in our experiment, however, only one high-potential cytochrome iron was reduced.

For the evaluation of the dynamic properties the total areas of the Mössbauer spectra were used. The values of the derived average mean square displacements, $\langle x^2 \rangle$, decrease linear with decreasing temperature below 180K. This behavior can be described by a Debye-law. For the RC-crystals the Debye-temperature is about 220K. Myoglobin-crystals[5] as well as freeze dried myoglobin[6] show an increase of flexibility above 200K. The additional increase of the $\langle x^2 \rangle$ - values above 200K, typical for biomolecules, is much smaller in the RC-crystals than in myoglobin: Between 200K and 250K membrane bound proteins seem to be less flexible than water soluble proteins.

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The chloroplast envelope: A model system for studying precursor-receptor interactions

Most chloroplast proteins are encoded in the nucleus and synthesized in the cytoplasm as precursor proteins bearing a transit peptide. The precursor proteins are transported through the envelope bilayer into the chloroplast. The first step during transport is the recognition of the proteins and the binding of the aminoterminal extension to the outer envelope membrane. The binding has been investigated using isolated envelopes and translation products of mRNA from greening cells and of hybrid released mRNA.

- The binding is specific for the precursors to chloroplast proteins like LHCP[1] and SSU[2]. The cell wall protein Thionin is not bound.
- The binding does not require energy in form of ATP and is independent of temperature in the range from 4°C to 37°C.
- The binding depends on the pH value and has two optima at pH 6.5 and pH 8.0. Gel electrophoretic analysis shows a higher binding capacity for SSU at pH 8.0 than at pH 6.5.
- The binding to increasing amounts of envelopes results in a saturation curve but not in a total binding of all in vitro translated chloroplast precursors.
- Studies of the kinetics of the binding reaction show an increase in the amount of bound proteins up to 7 min incubation time but no change in the pattern of bound proteins between 3.5 min to 20 min of binding.

1) LHCP = chlorophyll a/b binding protein
2) SSU = small subunit of ribulose-1,5-bisphosphate carboxylase

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M. Blumrich, E. Lücker and E. Petzinger

Effects of heavy metals and sulfhydrylgroup reagents on the transport of bile acids in isolated rat hepatocytes.

The uptake of bile acids from blood is exclusively mediated by liver parenchymal cells. Today, two bile acid transporter systems, a sodium dependent and a sodium independent one, are postulated (1). Taurocholate is the main substrate of the first one, while cholate is transported by both.

We investigated the effects of $CdCl_2$, $HgCl_2$, $ZnCl_2$ and Pb-Acetate on the uptake of ^{3}H-taurocholate and ^{14}C-cholate into isolated rat hepatocytes in cell suspensions. Besides Pb-Acetate, a dose dependent inhibition of bile acid uptake was observed in the range of 10-100 µM of the dissociated heavy metal salt. Since liver cells accumulated up to 350 fold cadmium, much higher local concentration of SH-group blocking heavy metals are expected within the cells.

In order to find a more specific inhibition the organic sulfhydryl reagents N-ethylmaleimide (NEM) and para-mercuribenzenesulfonate (PCMBS) were tested. The blockade of 100 µM PCMBS could be partly reversed by washing the cells, NEM blocked irreversibly. Dithiothreitol(DTT) reversed the PCMBS induced blockade nearly totally.

In summary SH-groups are in both bile acid uptake systems of significant relevance for substrate transport. So far in our hands none of the SH-reagents were able to block selectively only one system without affecting the other one.

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Kartierung und Charakterisierung eines Sucrose-Stoffwechselweges aus dem Escherichia coli Wildisolat EC3132

Die bekannten Sucrose-Stoffwechselwege in Enterobakterien sind ausnahmslos PTS-abhängig. Molekular gut untersucht sind die untereinander sehr ähnlichen Sucrose-Stoffwechsel, die bei Klebsiella pneumoniae chromosomal-kodiert (1) und bei pUR400 aus Salmonella typhimurium und Escherichia coli plasmid-kodiert (2) vorliegen. Außerdem wurde in einem E. coli Wildisolat ein chromosomal kodierter Sucrose-Stoffwechsel beschrieben, dessen Expression PTS-abhängig ist (3).

Der hier untersuchte Sucrose-Stoffwechsel in dem E. coli Wildisolat EC3132 dagegen ist eindeutig PTS-unabhängig und kartiert bei 51 min auf dem Chromosom.

Von Interesse ist dieser Stoffwechsel bei der Betrachtung der Entstehung der verschiedenen Sucrose-Stoffwechselwege in Bakterien. Das Konzept des Kollektiv-Chromosoms (4) besagt, daß lebensnotwendige Gene vorwiegend in stabilen Teilen der individuellen Chromosomen und variable Eigenschaften auf Transposons, Plasmiden und instabilen Bereichen der individuellen Chromosomen gefunden werden, von wo sie leicht durch horizontalen Gentransfer auch zwischen verschiedenen Arten ausgetauscht werden können (5).

Bei Untersuchungen zur Evolution von Stoffwechselwegen am Beispiel des Sucrose-Stoffwechsels sollte man abhängig vom Zeitpunkt des Gentransfers während der Evolution unterschiedliche Verwandtschaftsgrade und damit unterschiedlich aufgebaute Stoffwechselwege finden können, was z.B. die Lage der Gene auf dem Chromosom oder auf einem Plasmid und die PTS-abhängige bzw. unabhängige Expression dokumentieren. Dabei stellt der hier untersuchte Stamm EC3132 eventuell eine solche theoretisch geforderte Ausgangs- oder Zwischenform dar. Zu der dazu notwendigen molekulargenetischen Charakterisierung wurden deshalb die Sucrose-Gene kloniert und teilweise sequenziert.

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Hydroxylysine Glycosides in Bones from Patients with Osteogenesis Imperfecta (OI) and in Fetal Bone

Patients with OI suffer from extremely fragile bones and in some cases even die from this heritable disease. OI is caused by an abnormal structure of type I collagen which results in insufficient bone mineralization. Fibroblast cell cultures established from severely affected patients produce a type I collagen, which contains too many hydroxylated and glycosylated amino acids (4-Hydroxyprolin, 5-Hydroxylysin, β-D-Galactopyranosyl-(1->5)-hydroxylysin (GH), α-D-Gluco-pyranosyl-(1->2)-β-D-galactopyranosyl-(1->5)-hydroxylysin (GGH)). Increased amounts of these amino acids, which are formed by posttranslational modification of lysine and proline, have also been found in fibroblast cultures from healthy embryos[1].

Is type I collagen also overmodified in native bone? To answer this question we collected bone biopsies from OI-patients, age-matched healthy control persons and from fetuses. Type I collagen was prepared by demineralisation, enzymatic extraction and salt precipitation. The content of GGH and GH was measured by alkaline hydrolysis of proteins and quantitative amino acid analysis. For this purpose new chromatographic conditions had to be found. GGH was isolated from marine sponge and GH was prepared from GGH by mild acid hydrolysis[2].

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B. Böttcher, U. Lücken and P. Gräber

Structure of the Reconstituted ATP-Synthase from Chloroplasts

The structure of the isolated CF_oF_1 has been investigated by electron microscopy of negatively stained samples, indicating that the hydrophilic CF_1 part was connected via a stalk to the CF_o part. However, negative staining could lead to structural changes of the protein. Therefore, we have investigated CF_oF_1 reconstituted into asolectin liposomes by cryo-electron microscopy. With this method the proteoliposomes were rapid-

ly frozen in liquid ethane, and electron micrographs were taken at liquid nitrogen temperature. The image contrast results from the phase contrast of the electron waves traveling differently through water, lipids and protein; i.e., staining artefacts are excluded. It results that the CF_o part is integrated in the membrane and that the CF_1 part is connected by a stalk to the CF_o part.

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EPR- and ENDOR-Investigations of the VO^{2+}-substituted D-Xylose Isomerase from Streptomyces albus

D-Xylose isomerase (E.C. 5.3.1.5) catalyses the reversible isomerisation of α-D-xylose to α-D-xylulose and α-D-glucose to α-D-fructose. The enzyme from S. albus is a tetramer (M_r 172,500) of four identical subunits with M_r 43,100 as revealed by UV-Laser-Desorption-Mass-Spectroscopy (1).
The enzyme requires Mg^{2+} for its activity, which can be substituted by Co^{2+}. There are two Co^{2+} binding sites per monomer, the B-site with an octahedral and the A-site with a penta-coordinated or distorted tetrahedral structure, as characterized by EAS and MCD (2). Spectroscopically controlled exchange of Co^{2+} by other cations indicates that they occupy the Co^{2+} binding sites.
The binding of paramagnetic VO^{2+} was studied with EPR- and ENDOR-spectroscopy. Titration of the metal-free enzyme with VO^{2+}, monitored by EPR, shows that the first four VO^{2+} bind specifically to the B-site. The native Mg^{2+} enzyme is strongly inhibited in the presence of VO^{2+}. Frozen solution EPR-spectra of the B-site show typical anisotropic VO^{2+}-EPR signals with the parameters $g_\parallel = 1.942$, $g_\perp = 1.978$, $A_\parallel = 171 \cdot 10^{-4}\ cm^{-1}$, $A_\perp = 59 \cdot 10^{-4}\ cm^{-1}$. These results indicate that oxygens are the predominant ligands of this site (3).
More detailed informations are extracted from ENDOR measurements, a technique used for the first time on VO^{2+} bound to proteins to elucidate the ligand sphere of the metal binding sites. The nitrogen ENDOR-spectra in the range of 1-10 MHz show that at least one nitrogen is also involved in the binding of VO^{2+}. The proton ENDOR-spectra in the range of 10-20 MHz, recorded in H_2O and D_2O, reveal three protons in vicinity of VO^{2+}, two of them exchangeable.

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M.Boll

Profiles and Regulation of the Enzymes of Glycolysis in Spruce (Picea abies)

Activities of the glycolytic enzymes have been determined in seedlings,callus cell cultures and in cell suspension cultures of spruce.

The rate-limiting enzymes of the pathway appeared to be hexokinase(EC 2.7.1.1), fructokinase(EC 2.7.1.4),phosphofructokinase and pyruvate kinase (EC 2.7.1.40). Two phosphofructokinases(PFK)were detected:ATP-PFK(EC 2.7.1.11)and PP_i-PFK(EC 2.7.1.90).In the presence of its activator fructose-2.6-biphosphate, PP_i-PFK had a 2-3 fold higher specific activity than ATP-PFK.UDP-glucose-pyrophosphorylase(EC 2.7.7.9) and malate dehydrogenase(EC 1.1.1.37) were also found.

In seedlings,grown autotrophically with minerals all enzymes except fructose diphosphate aldolase (EC 4.1.2.13),glyceraldehydephosphate dehydrogenase(EC 1.2.1.12)and phosphoglycerate kinase (EC 2.7.2.3)had 50-150% higher activities in the hypocotyls than in the cotyledons,while the activity of the latter enzymes was higher in the cotyledons. This distribution remained unchanged up to 90d of cultivation of the seedlings.

In callus cell cultures and in green suspension cultures(light-grown on solid or in liquid medium,respectively, with sucrose as carbon source), which were derived from the seedlings, activity of most enzymes was 1.5-2.5 fold higher as compared with seedlings. Pyruvate kinase and phosphopyruvate carboxylase(EC 4.1.1.31) were 3-4 fold higher.

When autotrophically grown seedlings were aerated in liquid medium,enzyme activities increased within 1d to the levels found in callus cells and in the green suspension culture.This increase required the presence of a carbohydrate. It was completely prevented by 15 µg/ml Cyclohexiimide,thus indicating an enzyme induction.

In cell suspension cultures,enzyme levels tended to decline when,after longer periods of cultivation,carbohydrate became limiting.

These observations suggest a sensitive regulation of the enzymes of glycolysis by available carbohydrate in spruce.

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Andreas Borchard and Wolfgang Junge

The effect of pyridine on the onset lag of photophosphorylation: No evidence for "localized coupling"

For the protonic coupling mechanism of photophosphorylation it is still under debate whether protons are pumped into the aqueous lumen which serves as a reservoir for all ATPsynthases (eg. Mitchell [1961]) or whether protons are released within the membrane and travel in special intramembrane ducts to the ATPsynthase ("localized coupling", eg. Williams [1961]).
We investigated two chloroplast preparations which differed in their response to the buffer pyridine. In "high salt" thylakoids added pyridine enhances the onset lag of photophosphorylation in a series of light flashes, but it fails to do so in "low salt" thylakoids. Dilley and Coworkers [1986] interpreted this to indicate "delocalized" coupling in the former and "localized" coupling in the latter preparation.
We found that this behaviour corresponded to a different ability of pyridine to decrease the extent of flash light induced pH-transients in the lumen as indicated by absorption changes of neutral red. Only in "high salt" thylakoids addition of pyridine, up to 10 mM, caused a significant decrease of the transient signal, but not in "low salt" ones. In both preparations, contrastingly, tris buffer decreased the neutral red response and correspondingly enhanced the onset lag of phosphorylation. When pyridin was also added it further decreased the response, but again, only in "high salt" thylakoids.
We find that these effects reflect different access of pyridine to the lumen and we doubt that they give evidence for "localized" coupling.

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The Constitutive K^+-Transport System Trk from *Escherichia coli* is composed of Several Different Subunits

At K^+ concentrations above 200 µM in the medium cells of *E. coli* accumulate K^+ almost exclusively via the constitutive K^+-uptake system Trk. This system probably acts as a K^+-H^+-Symporter and has intriguing properties: i) its activity is regulated both by cell turgor and the cytoplasmic ATP level; ii) mutations in several genes scattered on the chromosome affect Trk activity. We will supply biochemical evidence that the 53.000 mol. wt TrkA protein is a periferal membrane protein, that requires at least the TrkG protein for its anchouring to the cytoplasmic membrane. We have identified TrkG as a 32.000 mol. wt membrane protein. TrkG aggregates when samples are boiled before SDS-PAGE. In gels run without urea TrkG form a very diffuse band, that becomes focussed upon inclusion of 6M urea in the gel system. This behaviour, typical for hydrophobic membrane proteins is consistent with TrkG being part of the integral membrane complex of the Trk system. The role of the TrkE and TrkH proteins has yet to be determined. The *trkD*-gene product forms a separate constitutive K^+-uptake system, Kup.

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T. Braulke, S. Tippmer, H. Damke and K. v. Figura

Regulation of cellular distribution of mannose 6-phosphate/IGF II receptors in human fibroblasts

The mannose 6-phosphate/insulin-like growth factor-II (M6P/IGF II) receptor is a multifunctional protein with specific binding sites for the M6P-recognition marker on lysosomal enzymes and IGF II. Most of the cellular M6P/IGF II receptors (80-90%) are localized inside the cell and function in the transport of lysosomal enzymes from the Golgi to the lysosomes. These receptors exchange continously with the remaining 10-20% present at the cell surface. Treatment of human fibroblasts with M6P, IGF I, IGF II, epidermal growth factor and insulin at 37°C causes a rapid redistribution of M6P/IGF II receptors from intracellular membranes to the cell surface. While the number of receptors increases up to 4-fold, the affinity of the M6P-binding sites is not affected. Dose response curves and experiments with blocking antibodies against the IGF I receptor revealed that IGF I and IGF II exerted their effects preferentially via their own receptor. The increase of cell surface receptors by growth factors is due to an increase of the externalization rate of preformed intracellular receptors. The effects of M6P and growth factors were additive while combinations of the growth factors were not additive indicating that M6P and the growth factors stimulate the redistribution of M6P/IGF II receptors by independent mechanisms.
To gain insight in the mechanisms mediating the receptor redistribution we studied components of second messenger systems: i) cholera toxin or pertussis toxin sensitive G_s- and G_i-proteins; ii) intracellular c-AMP concentration; iii) free cellular Ca^{2+}; iv) protein kinase C; v) arachidonic acid release and vi) the phosphoinositols. It became clear that G_s- and G_i-proteins and protein kinase C are involved in the control of the steady state concentration of cell surface M6P/IGF II receptors. Cholera and

pertussis toxin treatment and activation of protein kinase C abolished the M6P and growth factor stimulated receptor redistribution, respectively.
The increase in cell surface M6P/IGF II receptors led to an increased binding of IGF II and M6P-containing ligands and to an increased endocytosis of lysosomal enzymes. We conclude that the cellular distribution of M6P/IGF II receptors is regulated by multiple factors, which mediated their effects via different intracellular signalling pathways.

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Evidence for the existence of energy dependent excretion carriers in amino acid producing strains of *Corynebacterium glutamicum*

Several species of coryneform bacteria are used for the production of various amino acids, especially glutamate and lysine. Each of these amino acids is excreted specifically since no other one is detectable in considerable amounts in the culture supernatant. In order to obtain information about the nature of these transport processes we investigated the efflux of the amino acids glutamate, isoleucine and lysine, which are representatives for the major groups of amino acids, e.g. anionic, neutral, cationic. It was found, that in all three cases, amino acid excretion in *C. glutamicum* could not be explained in terms of a "leaky" membrane, which is in fact the generally accepted hypotheses in the literature[1]. We showed that cells of *C. glutamicum* fermenting one of the three different amino acids possess a normal membrane potential and maintain substantial substrate and ion gradients[2].
Glutamate is produced by wildtype cells under biotin limitation.
The efflux is strongly inhibited by CCCP (Carbonyl cyanide m-chlorophenylhydrazone) , but not by the addition of K^+/valinomycin, which specifically decreases the membrane potential. Glutamate can be excreted against an existing concentration gradient.
Isoleucine is produced by wildtype cells which are supplemented with the isoleucine precursor 2-ketobutyarate. Thus the feedback inhibition of isoleucine on its own synthesis is bypassed and the internal concentration of isoleucine increased.
Isoleucine can be excreted against an existing concentration gradient. K^+/Valinomycin, however, inhibits the excretion of isoleucine drastically while the internal concentration increases transiently because of the continuing intracellular synthesis[3].
Lysine is produced by strains of *C. glutamicum* in which the feedback inhibition by lysine is abolished by mutation or other genetic methods. The excretion is strongly affected by CCCP and valinomycin. Lysin has to be transported against the existing membrane potential.
We conclude that all three amino acids are excreted specifically in an energy dependent manner.The efflux is a carrier mediated process and the most important requirement for the excretion of these substrates seems to be a change in the metabolic conditions.

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Characterization of native and modified G-protein coupled receptors in microinjected oocytes

We have established that receptors for vasopressin (V1 subtype), angiotensin II, oxytocin, thyrotropin releasing hormone, bombesin and histamin can be fuctionally expressed and identified in X.laevis oocytes previously primed with poly(A) + RNA from the respective receptor containing tissue (1). Upon hormone application voltage-clamped oocytes showed concentration dependant oscillating whole cell current changes. In all cases the responses were specific since only cognate peptide but not unrelated hormone evoked changes in membrane current. The use of agonists and antagonists specific for a respective receptor triggered or blocked the response. Thus, Xenopus oocytes microinjected with the appropiate mRNA provide a valuable tool for the study of receptors linked to the inositol phosphate second messenger system (most probably via guanine nucleotide binding proteins).
The electrophysiological response in oocytes upon the application of an agonist can be used as an assay system which allows to monitor the functional state of a receptor. Using a receptor cloned into an in vitro transcription vector, the influence of modifications within the receptor gene on posttranslational modifications, ligand binding and activation of the second messenger system can be rapidly tested. We have used the rat serotonin HT2 receptor (2) and the human β_2-adrenergic receptor (3) as model systems.The influence of deletions and the exchange of sequence elements within the two receptors on their molecular properties will be described.

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Detection of a Possible Proton pathway and Light-Induced Structural Changes in Bacteriorhodopsin by Neutron Diffraction

In order to look for structural changes during the pumping cycle of bacteriorhodopsin (BR), we have obtained neutron diffraction patterns from oriented purple membranes containing BR exclusively in the ground state BR-568 or in the M-intermediate of the photocycle. Both, large scale global structural changes and rotational displacements of BR molecules in the unit-cell during the transition can be excluded. However, there are small but highly significant structural differences between both BR states as is revealed in the two-dimensional difference Fourier map calculated from structure-factor differences. In the M-state, there is a prominent density increase both in the vicinity of the cyclohexene ring and the Schiff's base end of the chromophore, which is interpreted by an approx. 2° tilt of 3 to 4 α-helices. As a second light-induced process, we have compared BR in the light- and dark-adapted states. A significant conformational difference in the protein moiety between these two states was not observed. By neutron diffraction measurements on purple membranes in the dark-adapted state at different H_2O/D_2O ratios, we have localized and quantified the distribution of water molecules and of exchangeable hydrogens in the membrane plane. The most exciting finding is a prominent density peak in the projected structure at the Schiff's base end of the retinal which might reflect the position of the proton pathway through the molecule. Part of this density maximum could be attributed to four molecules of water.

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Towards a Transformation Selection System Based on Nonsense Suppression in *Dictyostelium discoideum*

A system has been established to monitor transcription of individual tRNA genes in the cellular slime mold *Dictyostelium discoideum*. This system is based on nonsense suppression: The product of a mutated tRNA gene is able to read an integral translational stop codon within the message of a reporter gene, whereby a functional product is synthesized. The reporter gene is a hybrid gene construct between the *Escherichia coli* ß-galactosidase gene and the 5'-part of the actin 6 gene from *D. discoideum*. Cells carrying such a fusion gene stably integrated in their genome express functional ß-galactosidase. Variants of the reporter gene were constructed by point directed mutagenesis, rendering certain amino acid codons translational stop signals. From these genes active ß-galactosidase is only expressed if a corresponding suppressor tRNA is synthesized in the same cell. While UAG or UGA reading suppressor tRNAs are tolerated by the cells UAA reading suppressors are lethal. This observation seems to be rationalized by the extreme codon usage of *Dictyostelium*: UAA is used as translational stop signal in more then 95% of genes sequenced so far. Therefore an active UAA suppressor tRNA seems not compatible with cell growth.

Currently we are trying to set up a transformation selection system based on nonsense suppression. Variants of the neomycin phosphotransferase gene were constructed carrying gene internal translational stop codons. Cells containing such variant genes stably integrated in their genomes can only grow in the presence of the aminoglycoside G418 if a functional suppressor tRNA gene is expressed in the same cell. This suppressor tRNA gene is provided on a selection plasmid which can be constructed significantly smaller in size than conventional selection plasmids.

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Virus- versus endotoxin-induced Kupffer cell activation

Kupffer cells, the resident liver macrophages, are the key scavenger cells in the hepatic sinusoid to clear gut-derived microbial material. Upon exposure to endotoxin or virus, Kupffer cells respond with the release of molecules which may serve as signals for hepatocytes, other sinusoidal cells or peripheral blood cells.

We compared the release of lipid (PGE_2) and peptide (Interleukin-6 and Tumor Necrosis Factor-α) mediators after stimulation by endotoxin and virus, respectively. For viral induction we used Newcastle Disease Virus and Sendai Virus.

With respect to Interleukin-6 and Tumor Necrosis Factor-α, viral induction led to a much greater release of peptide mediators than endotoxin at a dose known to stimulate Kupffer cells maximally. Also the kinetics of cytokine release were different. Viral infection resulted in a much delayed response as compared to endotoxin stimulation.

With respect to lipid mediators, Kupffer cells exhibited also after viral induction the shift to predominant PGE_2 production, which is typically seen in response to endotoxin.

These studies show that viral induction leads to a higher state of activation of Kupffer cells than exposure to endotoxin.

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G. Busch and R. Tauber

Selective isolation of individual cell surface proteins by a cleavable biotin label

A novel method was developed to isolate cell surface proteins in a simple two-step procedure. Hepatocyte cell surface proteins were labeled by a cleavable biotin derivative (1) in a covalent pulse reaction. Under the described conditions, NHS-SS-biotin proved to be an impermeant, cell surface specific label which does not affect the viability of rat hepatocytes. Subsequent avidin affinity chromatography of hepatocyte proteins combined with a sulfhydryl reagent mediated elution under non-denaturating conditions, followed by alkylation of eluted proteins and submission to an immunoprecipitation with a monospecific polyclonal antibody, resulted in the isolation of a selected protein of the hepatocyte plasma membrane, gp120, documented as a single band in SDS-PAGE. Using the same technique, a transit time of gp120 from endoplasmic reticulum to the cell surface of 2 hours could be determined, a value related to that of the bulk of cell surface proteins (2).
The results indicate, that the described combination of labeling with a cleavable biotin derivative, non-denaturating avidin affinity chromatography and immunoprecipitation is a useful method to isolate and study individual cell surface proteins.

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Studies on the Superoxide Forming NADPH Oxidase in Human Granulocytes in a Cell-free System

Phagocytes contain a NADPH oxidase in the plasma membrane that generates superoxide anion (O_2^-), the key component in the oxygen-dependent antimicrobial mechanism. Upon activation of the oxidase the turnover of phospholipids seems to play an important role. Thus activation is achieved by cis-unsaturated fatty acids like arachidonic acid and a high molecular mass cytosolic factor(1). We investigated this mechanism in subcellular fractions of granulocytes in order to study patients with a defect in the production of superoxid ("chronic granulomatous disease")(2).
In our modified preparation method anticoagulated blood was layered on Ficoll-Hypaque and the granulocytes were sedimented by centrifugation. The erythrocytes were removed by treatment with isotonic NH_4Cl. The granulocytes were disrupted by sonication and the sonicate was applied on a discontinuous Percoll gradient. The cytosolic fraction and the plasma membrane were collected and purified by high speed centrifugation.
By combining the subcellular fractions, we can localize the defect in patients. We investigated a patient, who seems to show a variant of chronic granulomatous disease, i.e., who has nearly normal monocytes but defective granulocytes. This was established by criteria as superoxid production, reduction of nitrobluetetrazolium, chemiluminescence, bactericidal capacity and glucose oxidation. We localized the defect in the plasma membrane of the granulocytes, not in the cytosolic fraction or in the activation mechanism dependent on arachidonic acid.

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Isolation of a Cytoplasmic Thioltransferase from Bovine Kidney

The enzymatic activity of glutathione-disulfide transhydrogenase (thioltransferase, EC 1.8.4.2) has been characterized in E. coli in comparison to thioredoxin. It has a molecular mass of about 10 kDa and has been named glutaredoxin(1). In mammals an enzymatic activity of about 55 kDa was found in microsomes and has been called glutathione-insulin transhydrogenase or protein disulfide-isomerase(2). The cyto-

solic enzyme has only been characterized from liver and thymus[3]. In order to determine, if the enzyme exists also in other mammalian tissues besides thioredoxin, we isolated the enzyme from bovine kidney[4]. As purification steps we used heat treatment, acetone fractionation and chromatography on DEAE-Sephadex, Sephadex G-75, hydroxylapatite and Sephadex G-50. The highly purified enzyme showed only bands of a molecular mass of about 12 kDa in SDS gel electrophoresis, no bands with a molecular mass of thioredoxin reductase (two subunits of 58 kDa). The enzyme shows the typical behaviour of glutathione-disulfide transhydrogenase, i.e. the activity depends on glutathione, glutathione reductase and NADPH. Cystine is used as substrate for the enzyme assay. The enzyme can accept high molecular mass substrates like oxytocin, insulin and trypsin.

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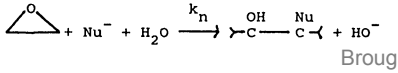
Reactivity and Stability of Substituted Oxiranes

Epoxides are formed in vivo from alkene precursors. The mutagenic and oncogenic potential of these compounds is related to their reactivity and stability in the aqueous environment of the organism[1]. The reactivity of 1,2,3,4-diepoxybutane, 1,2-epoxy-3-butene, 1,2-epoxy-2-methylpropane and 1,2-epoxipropane was determined on the basis of their reaction rate with different nucleophiles (I^-, Br^-, Cl^-, $S_2O_3^{2-}$ and SCN^-) by measuring the OH^- production from the chemical reactions[1]:



(1)

The epoxides were reacted with the nucleophiles in a pH-stat, at 25°C, pH = 7.15, under a nitrogen atmosphere. 0.03N HCl was used to neutralize the OH^-.

An extrathermodynamic approach proposed by Swain and Scott[2] was used to estimate the reactivity of the epoxides according to:

$$\log \left(\frac{k_n}{k_0}\right) = s*n \qquad (2)$$

where

- k_n the second order rate constant for the reaction with a given nucleophile
- k_0 the second order rate constant for the reaction with water
- s the susceptibility, as a measure for the reactivity of the epoxide
- n the nucleophilic parameter, specific for the nucleophile

The reactivity parameters (k_0 and s) were determined from equation (2) with the least square method.

Based on the s-values the relative reactivities of the epoxides decrease in the following order: 1,2,3,4-diepoxybutane > 1,2-epoxy-3-butene > 1,2-epoxy-2-methylpropane > 1,2-epoxypropane. The relative reactivites of the epoxides against biologically relevant nucleophiles e.g. aminoacids, glutathione and guanosine are also described.

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Metabolic Transformation of Substituted Olefines - a Theoretical Chemical Approach

Olefinic hydrocarbons are metabolized by Cytochrome P-450 enzymes to expoxides, which may be mutagenic and carcinogenic[1]. A model was deve-

loped which describes the metabolic transformation rate of ethene, 1-fluoroethene, 1,1-difluoroethene, 1-chloroethene, 1,1-dichloroethene, cis-1,2-dichloroethene, trans-1,2-dichloroethene, 1,1,2-trichloroethene, perchloroethene, propene, isoprene and 1,3-butadiene on the basis of molecular parameters of these compounds. To evaluate the model, metabolic rate constants, experimentally determined by inhalation pharmacokinetics (using a two compartment model) were taken from the literature[2]. Metabolic epoxidation is described as an electrophilic reaction of the olefine. π-Electron densities, normalized by the HOMO-energies, were used as a measure for the different reactivities of the compounds. The HOMO-energies and π-electron densities were calculated with the MNDO-method[3]. The HOMO-energies were corrected by including the ionization potentials into the calculation. Our model is described by:

$$k_i^{app} = \frac{a^* \mu_i * d(\pi)_i}{Ip_i - b} + c \qquad (1)$$

k^{app} - Velocity constant of metabolism $[h^{-1} \cdot kg^{-1}]$ under conditions of metabolic saturation (metabolism saturated to at least 90 %).

$d(\pi)_i$ - π-Electron densitiy at the carbon atom, with the highest π-electron density.

μ_i - Dipolmoment.

Ip_i - Ionization potential.

With a = 0.011 ± 0.001, b = 9.730 ± 0.001 and c = 0.008 ± 0.005 as parameters, specific for this class of compounds, our model gives a good correlation (R = 0.9517) with the experimental data.

Our results show, that molecular parameters of substituted ethylenes can be used to predict the metabolic transformation rates of these compounds.

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Identification of interleukin-6 responsive elements in the promoter region of the rat α_2-macroglobulin gene

The synthesis of α_2-macroglobulin (α_2M), the major acute phase protein in the rat, is induced by recombinant human interleukin-6 (rhIL-6) in rat hepatocyte primary cultures as well as in the rat in vivo. In order to investigate the mechanisms of α_2M-induction, we have isolated the rat α_2M-gene and characterized its 5'-end (1). Chimeric plasmids containing 1.3 kb of the 5'-flanking region as well as several deletions were linked to the gene for the bacterial chloramphenicol acetyltransferase (CAT) and introduced into human HepG2 cells. After stimulation with rhIL-6 CAT activity was inducible, even without dexamethasone. Deletion of 469 nucleotides from the 1.3 kb 5'-flanking region had essentially no influence on the expression of CAT activity after IL-6 stimulation. Further deletion of 75 nucleotides (-852 to -777), led to a drastic loss of CAT activity, indicating the presence of a cis-acting element. Complete loss of CAT activity was observed after deletion of sequences between -336 and -165, reflecting the existence of a second cis-acting element. Computer analysis led to the identification of the sequence 5'-CTGGGA-3' (-170 to -165), earlier described by Fowlkes et al (2) as acute phase consensus sequence. A synthetic oligonucleotide (-176 to -159), containing this sequence, was linked to the SV40 promoter and the CAT gene. After transfection into HepG2 cells CAT activity could be induced by IL-6. Mutations in this sequence resulted in a loss of CAT activity or in the complete loss of IL-6 inducibility. This observation clearly shows that the sequence 5'-CTGGGA-3' is an acute phase responsive element.

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Glucagon- und beta-adrenerge Ansprechbarkeit der Rattenleber

Untersuchungen zur Glucagon- und beta-adrenergen Ansprechbarkeit der Leber wurden an isolierten Hepatocyten und Leberhomogenaten fetaler und adulter Ratten verschiedenen Alters und beiderlei Geschlechts durchgeführt.
Folgende Ergebnisse wurden erhalten:

1. Maximale Stimulierung der Glucosefreisetzung wurde unabhängig von Geschlecht und Alter bei 10^{-7} M Glucagon erreicht.

2. Glucagon und Isoproterenol stimulierten die Adenylatcyclase in Hepatocyten fetaler Ratten auf 237 bzw. 232%. In Hepatocyten adulter weiblicher Ratten wurden für Glucagon 716-834% und für Isoproterenol 186% gemessen. Bei männlichen Ratten hatte Isoproterenol keinen Effekt.

3. Halbmaximale Stimulierung der Adenylatcyclase und der Glucosefreisetzung wurde bei Glucagonkonzentrationen von 10^{-7} M bzw. 10^{-10} M erzielt.

4. An Rohmembranpräparationen von adulten Hepatocyten wurden 106 fmol/mg EW von fetalen Hepatocyten 36 fmol/mg EW ^{125}I-Glucagon gebunden. Der Anteil hochaffiner Bindungsstellen in adulten Ratten ist höher als in fetalen. Die beta-adrenerge Bindung ermittelt mit ^{3}H-CGP 12177 war 34.3 fmol/mg EW bei fetalen, 15.1 fmol/mg bei 10 Monate alten männlichen und 15.5 fmol/mg bei weiblichen Ratten.

5. Der Phorbolester TPA verminderte die Glucagon Stimulierung der Adenylatcyclase adulter Hepatocyten um 30 %, ohne die Stimulierung durch Guanylylimidodiphosphat und Forskolin zu beeinflussen.

6. Unter in vivo-Bedingungen war der cAMP-Spiegel zum Zeitpunkt der Geburt durch Glucagon nicht stimulierbar. Isoproterenol jedoch erhöhte den cAMP-Spiegel um 130 %. Zur 2. und 6. Stunde post partum erhöhten beide Hormone signifikant den cAMP-Spiegel um 131-184%.

Die Ergebisse werden diskutiert hinsichtlich alterabhängiger Veränderungen der hormonalen Ansprechbarkeit sowie der Glucagonresistenz der Rattenleber unmittelbar nach der Geburt.

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Specific Labeling of a 55-kDa Protein from Rat Renal Brush Border Membranes with ^{3}H-Azidophlorizin

Phlorizin is a specific high affinity inhibitor of the Na^+/D-glucose cotransporter in intestinal and renal brush border membranes (BBM) with a K_I of about 1 µM. Azidophlorizin (4-desoxy-4-azidophlorizin) can be used for irreversible inactivation of the Na^+/D-glucose cotransporter after irradiation at 254 nm. We, therefore, synthesised tritium-labeled azidophlorizin with a specific activity of 18 Ci/mmol and tried to label the Na^+/D-glucose cotransporter in rat renal BBM after solubilisation in CHAPS. Eight major labeled protein bands, ranging from 25 to 200-kDa, could be observed after irradiation in the presence of 0.5 µM ^{3}H-azidophlorizin, SDS-PAGE and autoradiography. Labeling of the 25- and 55-kDa protein bands was clearly reduced, when the solubilised BBM was first irradiated in the presence of 0.2 mM non-radioactive p-azidophenyl-ß-D-glucoside. At this concentration the glucoside irreversibly inactivates the Na^+/D-glucose cotransporter.
Affinity chromatography with phlorizin columns (3-aminophlorizin coupled to different column materials) allows the specific enrichment of a 55-kDa protein from rat renal BBM solubilised with CHAPS. Investigations are under way to determine wether the labeled and the affinity-purified 55-kDa protein are identical and are (part of) the renal Na^+/D-glucose cotransporter.

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T. Dick and H. Matzura

Translational Attenuation: An in vitro Analysis

The chloramphenicol (Cm)-inducible cat gene derived from the Staphylococcus aureus plasmid pUB112 encodes a Cm-acetyltransferase (CAT, EC 2.3.1.28). In vivo studies using Bacillus subtilis suggested a translational regulation mechanism (1,2,3,4). According to the model a Cm-bound ribosome translating the leader mRNA stalls at a leaderpeptide-directed site, where it disrupts the base-pairing of a leader anti-Shine-Dalgarno sequence with the cat ribosome binding site, thus allowing initiation of CAT synthesis. We have used a B. subtilis in vitro translation system and in vitro synthesized cat mRNA to test the model. We showed that induction of CAT synthesis in vitro is post-transcriptional. Cm has no significant effect on the stability of the cat mRNA in vitro, and hence the post-transcriptional induction occurs by stimulation of translation. The induction effect of Cm could be mimicked by using a cat mRNA derivative lacking the leader sequence up to the cat ribosome binding site. These results suggest a Cm-dependent shift from an attenuated leader mRNA structure to a translational active conformation and could be explained satisfactorily by the translational attenuation model.

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Yeast mutant defective in peroxisome assembly

Methylotrophic yeasts like Hansenula polymorpha develop large peroxisomes when grown on methanol as the sole source of carbon and energy. The major peroxisomal enzyme methanol oxidase (EC 1.1.3.13.) constitutes a regular electron-dense crystalloid structure within the organelles. Overproduction of methanol oxidase by transformation with several copies of the cloned gene[1] was accompanied by an increase in peroxisome size and total synthesis of the enzyme at amounts of 60% based on the total soluble protein[2]. A mutant affecting methanol oxidase activity was isolated

from such transformants showing less than 5% of enzyme activity compared to transformed cells. Analysis of crude extracts by SDS-PAGE showed severe proteolytic fragmentation of peroxisomal proteins. No regular peroxisomes were detected in mutant cells by electron microscopy. Instead, electron-dense clusters that probably represent aggregates of peroxisomal proteins were observed.
This strategy of mutant isolation and characterization will be helpful for the identification of factors required for peroxisome assembly.

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T.Dierks and R.Krämer

Chemical modification of the mitochondrial aspartate/glutamate carrier. Effects on antiport and unidirectional transport function

The Asp/Glu carrier was investigated in the reconstituted system[1,2] consisting of liposomes that carry enriched carrier protein[3] of definite transmembrane orientation[2]. The kinetic mechanism of antiport was characterized as a concerted transport of internal and external substrate involving one substrate binding site on each membrane side[4]. In order to gain insight into structural aspects of this transport process, specific amino acid residues of the carrier protein were modified.

After treatment with DTNB[2] and NEM the antiport activity was strongly reduced and could be blocked completely by mercurials. Interestingly, several mercury compounds were able to convert the antiporter into a unidirectional efflux carrier[5]. This change could completely be reverted by treatment with dithioerythritol. Efflux activity could clearly be related to the Asp/Glu carrier, although in the efflux state some basic properties were different from the antiport state. The most striking difference was the drastically reduced substrate specificity and transport affinity of the carrier. These results suggest that the externally applied mercurials affect the internal binding site leading to a somewhat unspecific flux through the transmembrane channel of the carrier protein. This process, contrary to the antiport, was very sensitive to osmotic gradients across the liposomal membrane.

In order to achieve decoupling of antiport at least two reactive cysteines had to be modified. If the SH-reagent only had excess to one class of cysteines, an intermediate state of the carrier could be described showing neither antiport nor efflux activity. The switching between the two functional states was restricted or stimulated by other reagents such as pyridoxal phosphate, palmitoyl-CoA or polyanions.

Furthermore, the reconstituted carrier protein was modified by hydrophilic carbodiimides, which inhibited the antiporter completely suggesting that some essential carboxyls are involved in antiport function. The action of the carbodiimides could specifically be reduced by low concentrations of aspartate or glutamate. The nature of this substrate protection as well as the consequences of this modification for the induction of efflux activity were investigated.

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Spectroscopic studies on the di-iron center of the purple bovine spleen phosphatase

The purple acid phosphatase with a novel binuclear metal center is a lysosomal enzyme (1) which in the physiological surrounding acts in the reduced Fe(II)-Fe(III) state. After preparation in presence of air the enzyme is obtained in the oxidized Fe(III)-Fe(III) form. It contains one very strongly bound phosphate ion and has an absorption maximum at 540 nm due to a tyrosine-Fe(III) charge-transfer-transition. Phosphate can be removed only after reduction and separation of the enzyme by gel filtration. Reoxidation by 1.5 eq. of H_2O_2 or peroxodisulfate in Bis-Tris-buffer leads to a Fe(III)-Fe(III)-species absorbing now at 558 nm. Addition of acetate or oxidation in acetate buffer shifts the maximum to 550 nm. Addition of 1 eq. phosphate to both species shifts the absorption immediately to 540 nm. EXAFS data show that this enzyme species is identical with the species as prepared. It is suggested that bridging occurs by μ-oxo, acetato and phosphato groups respectively. Addition of other oxoanions leads to species with absorptions ranging from 557 nm (nitrate) to 533 nm (wolframate) depending on the distance of the bridging oxygens. The same dependence can be found for the coupling constants in the susceptibility measurements due to antiferromagnetic spin coupling of both Fe(III).
The reduced enzyme has an absorption maximum at 515 nm at pH higher than 4 with a shift to 524 at lower pH values. Saturation with phosphate leads to a pH dependent species shifting from 519 nm at pH 6.1 to 560 nm at pH 3.1 as also seen in uteroferrin (2).

From the pH-dependence of the reaction and of these spectral data including that of the ESR-signal (3) and other published data we propose a mechanism (4) where the hydroxo ligand of Fe(III) attacks the substrate bound by electrostatic interaction to the Fe(II), which also can be replaced by other transition metal cations (see Körner, Suerbaum and Witzel)

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J. Dreher and H. Matzura

Deletion-analysis of a Staphylococcus aureus plasmid in Bacillus subtilis

The naturally occuring S. aureus plasmid pC223 shows like most of the other S. aureus plasmids in B. subtilis segregational instability (seg. rate is 0.64%) and a decreased copy number (9/cell), which might be caused by an alternative partitioning and/or copy-number regulation mechanism in B. subtilis leading to dysfunctional host-plasmid interaction (1,2). Subject of this work was to study the behaviour of pC223-deletion derivatives in B. subtilis. The deletion derivative pJDD16 which lacks two DdeI fragments outside of the basic replicon is more stable (seg.rate is 0.34) than the wild-type plasmid and has an increased copy number (23/cell) in spite of their equal basic replicons. pJDD12, a second deletion derivative, lacks an additional DdeI fragment with essential elements of plasmid replication control: i. the region of the two negative regulating copRNAs, ii. both repM-promoters and iii. the first 36 nucleotides of the repM-coding region. This plasmid is inherited with a high copy number (46/cell) and shows an increased segregational instability (seg.rate is 0.83%).
Possible mechanisms which lead to this altered behaviour of the deletion plasmids in B. subtilis are discussed.

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K.Düring and S.Hippe

Synthesis, assembly and targeting of foreign chimeric proteins in transgenic *Nicotiana tabacum* cells

Secreted proteins normally contain a N-terminal signal peptide which directs insertion of the newly synthesized protein into the lumen of the endoplasmic reticulum (ER). During passage of the ER membrane the signal peptide is cleaved off.
In transgenic plants protein targeting and assembling of multimeric foreign proteins form an essential feature of gene technology. First, the secretion of a small procaryotic protein fused to a plant signal peptide in transgenic *Nicotiana tabacum* was investigated; second, the expression of light and heavy chain of a monoclonal antibody (each fused to the same signal peptide) in transformed tobacco cells was expected to result in the assembly of a biologically active antibody.
Chimeric genes were constructed using the coding sequence for the barley aleurone α-amylase signal peptide which was fused to the bacteriophage T4 lysozyme gene, the light and the heavy chain of a monoclonal antibody, resp. *Nicotiana tabacum* plants were stably transformed either by the lysozyme or by the light and heavy chain chimeric genes.
The chimeric lysozyme gene is expressed in transgenic tobacco cells to produce a precursor protein which is processed *in vivo* as shown by Western blotting. By electron microscopic immunogold labeling the transport of the lysozyme from the tobacco cells to the intercellular spaces could be demonstrated.
Synthesis and assembly of the monoclonal antibody in transformed plant tissue could be shown by "Tissue printing" and Western blotting. Purification by affinity chromatography on matrix bound haptene revealed biological activity of the produced antibody.

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The dehydration of (R)-2-hydroxyglutaryl-CoA to glutaconyl-CoA by an enzyme system from Acidaminococcus fermentans. The detection of a free radical

The dehydration of (R)-2-hydroxyglutaryl-CoA to glutaconyl-CoA is a key step in the fermentation of glutamate by the strict anaerobic eubacterium Acidaminococcus fermentans. The reaction is catalysed by an extremely oxygen-sensitive enzyme system requiring catalytically amounts of ATP. A component thereof was puri-

fied and characterized as a [FeS] protein[1]. The structural genes of its subunits (α, M = 53,870 kDa and ß, 41,857 kDa) were cloned in E. coli and sequenced. The derived aminoacid sequences do not show any homology to other enzymes although some similarities to nitrogenase could be detected[2]. The substrate of the dehydratase is generated during the assay from acetyl-CoA and (R)-2-hydroxyglutarate catalysed by glutaconate CoA-transferase (EC 2.8.3.12). However, HPLC analysis indicated the production of two isomers, the 1- and 5-CoA esters. The former was synthesized independently via the CoA ester of butyrolacton-5-carboxylate followed by acid hydrolysis. Only the 1-isomer is converted to glutaconyl-CoA by cell-free extracts from A.fermentans.
EPR-spectroscopy of the purified [FeS]protein revealed two different signals. The signal with an optimum temperature at 13K resembled that of a [3Fe-4S] cluster. It was not saturable by increasing the microwave energy. The other signal visible between 4 and 77K was saturable and most likely that of a free organic radical. It was quenched after exposure of the [FeS] protein to air, whereas in the presence of dithionite both signals were lost.

Since the dehydration of (R)-2-hydroxyglutaryl-1-CoA occurs against the rule of Markownikoff, the radical should be involved in catalysis.

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P. Eckard and H. Passow

Membrane Protein Phosphorylation Coupled to Sodium-Independent Phosphate Transport in Xenopus Oocytes

Previously, Na^+-dependent and Na^+-independent phosphate transport systems in the plasma membrane of Xenopus laevis oocytes have been described [1]. The former leads to accumulation of P_i in the cytosol, the latter to incorporation of P_i into a membrane fraction. These processs have been further investigated.
The Na^+-independent transport shows a linear time dependence for at least 3 hours. It consists of a saturable and a non-saturable component. The non-saturable component becomes apparent only at P_i concentrations above 25 µM. The saturable component is inhibited by p-chloromercuribenzenesulfonic acid (PCMBS).
After Na^+-free incubation of oocytes with $^{32}P_i$ (25 µM P_i) for 1 hour more than 80% of the radioactivity is incorporated into a membrane fraction which can be precipitated by trichloroacetic acid (TCA). Two phosphoproteins (M_r > 250 kDa) containing ≈ 14% of the transported $^{32}P_i$ were detected by SDS-PAGE and autoradiography in the membrane fraction. The presence of sodium during incubation neither leads to additional phosphorylation of the membrane fraction nor of the phosphoproteins although the amount of $^{32}P_i$ in the oocyte is greatly increased by Na^+-dependent transport. Similiarly the Na^+-dependent transport as observed after inhibition of the Na^+-independent transport by PCMBS did not supply phosphate for the phosphorylation reactions.
The phosphoproteins may be dephosphorylated in vitro by the addition of Mg^{++} (K_m ≈ 3 mM). This process is partly inhibited by PCMBS.
We conclude that in contrast to the Na^+-dependent P_i-uptake the Na^+-independent P_i-transport leads to phosphorylation reactions in a specialized membrane compartment.

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Trehalosestoffwechsel in *Escherichia coli*

E. coli kann auf Trehalose als einziger Kohlenstoffquelle wachsen. Es existiert eine periplasmatische Trehalase, welche Trehalose in zwei Glukosemoleküle spaltet und deren Gen, *treA*, bei 26 min. kartiert. Dieses Enzym ist teilkonstitutiv und durch hohe Osmolarität induzierbar.
Eine *treA-ptsG-ptsM*-Mutante wächst jedoch bei niedriger Osmolarität noch auf Trehalose. Folglich muß es einen zweiten Stoffwechselweg geben. Mutagenese eines *treA*-Stammes erlaubte die Isolierung verschiedener Trehalose$^-$-Phänotypen:
UE 46 trägt eine *lacZ*-Operonfusion. UE 46 transportiert Trehalose, wächst aber nicht auf diesem Zucker. Die Fusion liegt in einem Gen für den cytoplasmatischen Trehaloseabbau und wird bei hoher Osmolarität reprimiert.
Krim 3 wächst nicht auf Trehalose und reprimiert die Expression der *lacZ*-Fusion aus UE 46, betrifft also ein regulatorisches Protein.
Krim 4 zeigt weder Trehalosetransport noch Trehalosewachstum und wurde als Transport$^-$-Mutante charakterisiert.
Die Mutationen in Krim 4 und UE 46 sind kotransduzierbar, kartieren bei 96 min. und werden durch ein klonierten 12 kB DNA-Fragment komplementiert. Die Mutation in Krim 3 kartiert bei 52 min.
Mutanten, die zur konstitutiven Expression der Fusion bei hoher Osmolarität führen, zeigen keinen konstitutiven

Trehalosetransport. Die für Transport und Stoffwechsel notwendigen Gene sind also kotransduzierbar, aber nicht gemeinsam reguliert.
Die bisherigen Ergebnisse sprechen für folgendes Modell: Die Trehalose gelangt über ein spezifisches Phosphotransferasesystem ins Cytoplasma. Das dabei entstehende Trehalose-6-Phosphat wird durch eine bisher noch nicht genauer charakterisierte Phosphatase dephosphoryliert und die Trehalose durch ein cytoplasmatisches Enzym abgebaut, das zur Spaltung in Glukose und zum Aufbau längerkettiger Dextrine führt.

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Ornithine-Oxoacid-Aminotransferase Catalysed Turnover of Difluoromethylornithine: a Possible Contribution to the DFMO-Insensitivity of *Tetrahymena*

Difluoromethylornithine (DFMO), the enzyme activated irreversible inhibitor of ornithine decarboxylase (ODC, EC 4.1.1.17), has been used to block the growth of numerous eucaryotic cells and organisms due to polyamine depletion[1]. This strategy has been successfully employed to cure Trypanosomal (e.g. Sleeping Sickness) and Plasmodia (malaria) infections because these protozoa were very sensitive to that drug[2].
Another protozoon, the nonpathogenic ciliate *Tetrahymena thermophila*, has been shown to be insensitive to DFMO because normal growth is observed in the presence of 20mM DFMO in the medium, even in combination with MGBG, the enhancer of DFMO effects. Previous reports on ODC in crude extracts from *T. thermophila* suggesting complete resistance to DFMO[3] could not be verified with the pure enzyme (Eichler, W. *J. Protozool.* in print) because the inhibition was shown to be competitive with a K_i of 0.15 (which is in the order of magnitude of the K_m=0.11mM for the substrate, L-ornithine). Although the inactivating effect was less pronounced than with ODCs from other sources, this can not be the sole reason for the failure of DFMO to impair the growth of *Tetrahymena.*
After a short time lag, putrescine and spermidine concentrations in *Tetrahymena* cultures with 20mM DFMO were equal to those in control experiments, although the DFMO concentration had been decreased to ca. 14mM within 18h as determined by amino acid analysis. Partially purified ornithine-oxoacid-transaminase (OδT, EC 2.6.1.13) from *Tetrahymena*[4] was capable of producing an o-aminobenzaldehyde positive substance[5] from DFMO, thus suggesting a degradation of the ornithine analogue by the abundant enzyme[6].
Two mechanisms are discussed for the low in vivo sensitivity of the ciliate to DFMO: 1. *Tetrahymena* can degrade DFMO enzymatically by OδT keeping its intracellular concentration below the threshold of 2mM for the inactivation of *T. thermophila*-ODC. 2. *Tetrahymena* can increase the intracellular ODC activity to the 200 fold if required, possibly utilizing an amplified ODC-gene in the macronucleus, thus compensating a possible loss due to inactivation.

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W. Eichler

Polyamine Biosynthesis in the Ciliate *Tetrahymena thermophila*: Control by Putrescine and Spermidine

The requirement for polyamines, as expressed by growing or resting state, governs their biosynthesis as well in procaryotes as in eucaryotic cells: following growth stimulation, the regulative key enzyme of polyamine biosynthesis, ODC (L-ornithine decarboxylase, EC 4.1.1.17), is stimulated with growth and inactivated upon polyamine excess. This regulative scheme has also been verified in the ciliate *Tetrahymena*[1] as in numerous other eucaryotes[2]. However, the regulation of polyamine biosynthesis in this protozoon is also achieved on the level of substrate supply. L-ornithine is produced from L-arginine in this particular organism on a two step pathway involving the enzymes L-arginine iminohydrolase (ADI, EC 3.5.3.6) and citrulline hydrolase (CH, not yet registered)[3] and ornithine concentration is controlled by a competitive inhibition of ADI by ornithine and by degradation of this compound by L-ornithine-oxoacid-aminotransferase (OδT, EC 2.6.1.13).

Studies on the interactions of the polyamines putrescine (Put) and spermidine (Spd) which are abundant in rapidly growing *Tetrahymena* cells (14mM Put, 4mM Spd)[4], revealed that these substances were potent incompetetive inhibitors of ADI (K_{ii} of 2.8mM for Put and 4.3mM for Spd). Addition of Put and Spd to stimulated cultures suppressed the increase of ODC activity as expected, but also the stimulations of ADI and CH were impaired appreciably.

These results give more evidence for the involvement of the Arg degradative pathway of *Tetrahymena* in polyamine biosynthesis.

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W. Eichler and A. Münch

Citrulline Hydrolase from *Tetrahymena thermophila*: a Diagnostic Tool for the Prevention of Citrullinemia

Citrullinemia is not a common disease in Europe and Northern America, but in Asia, especially in Japan, familiar citrullinemia is relatively frequent. Diagnosis of this hereditary disease, which is expressed in patients lacking functional argininosuccinate synthetase, is very easy because, besides the increased citrulline concentration (0.5 to 1mM), the ammonia concentration is also increased dramatically by the block in the urea cycle. Ammonia intoxication is the reason for the serious consequences like brain dysfunctions and the common disorders, leading to an early death of the patients[1]. The completely lacking activity of argininosuccinate synthetase is observed only in patients who are homozygous for this enzyme gene defect; heterozygous persons are apparently healthy, because brain function and blood ammonia levels are normal. However, the citrulline concentrations in blood are increased to 200 to 300µM whereas the upper limit is 100µM for healthy controls. Although this increased citrulline level is not a pathological state, it is an indicator for the impaired gene function, and the children of a heterozygous couple will be homozygous with a probability of 0.25. Therefore, a screening assay for the detection of increased citrulline concentrations would be of great value. Since previous methods for the quantitation of citrulline have been either susceptible to disturbance by urea, like the colorimetric assay[2], or have been very time consuming, e.g. amino acid analysis, an enzymatic assay has been developed[3,4] based on the enzyme citrulline hydrolase from the ciliate *Tetrahymena thermophila*[5,6]. The major drawback of this method, the insufficient stability of the enzyme, has been eliminated by modifying the preparation procedure and two new assay protocols have been tested which appreciably decrease the assay time per sample, thus allowing large-scale screening studies. The results of these improvements of the method are given in this contribution.

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W. Eichler and B. Schertel

Arginine Starvation May Cause Diabetes Mellitus in Rats

Omitting the essential amino acid arginine from diets of rats alone, will result in multiplying the production of orotic acid, a pyrimidine base precursor in these animals[1]. Alloxan, another pyrimidine derivative, is frequently used to produce experimental diabetes mellitus in mammals. As a mechanism for the toxic action of this substance it has been claimed that alloxan is capable of producing oxygen radicals within the cell, toxic compounds, which cause calcium efflux from mitochondria[2], possibly by inducing ADP-ribosylation of the mitochondrial Ca^{2+}-channel[3].

The enzyme dihydroorotate dehydrogenase (DHO-DH, EC 1.3.3.1) which is located at the outer surface of the inner mitochondrial membrane, catalysing the conversion of dihydroorotate (DHO) into orotate, has been reported to produce cytotoxic radicals as well[4]. We were able to show that DHO, like alloxan, is able to cause Ca^{2+}-efflux from energized intact rat liver mitochondria, and that for both substances the effect is due to the action of DHO-DH, because by inhibition of this enzyme, radical formation and calcium-efflux could be diminished[5].

Calculations of the relative potencies of the two pyrimidines have shown that the overproduction of orotate, as induced in rats by arginine starvation, might be sufficient to cause diabetes mellitus in rats. However, in our experiments no clearly diabetic animal was detected. Possibly, combination with other noxious occurrencies, e.g. infections, hypoxia, ischemia, is required to enhance the DHO-effect. More experiments with arginine starved rats, treated with other damaging agents in addition, may give an answer to this still open question.

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Hormonally controlled uptake of L-glutamate in primary cultures of bile-duct epithelial cells

Hepatocytes are characterized by a Na^+-dependent transport system for L-glutamate (system G^-) which is strongly inducible by dexamethasone [1]. It has been assumed that

this transport system may serve to reabsorb glutamate from bile [2]. We wanted to investigate whether bile-duct epithelial cells (BEC) could be involved in this reabsorption process. We have therefore determined the capacity for transporting glutamate in isolated and cultivated BEC.
BEC were isolated as a pure cell population by enzymatic dissociation of liver tissue by collagenase/hyaluronidase followed by an incubation of remaining tissue with trypsin. The cells were cultured in DMEM up to 96 h. Uptake was measured for 1 to 5 min in sodium or choline containing transport medium [1].
The uptake of glutamate into BEC after 24 h of cultivation (240 pmoles/min/mg) was very high compared to uptake in hepatocytes and even increased during cultivation to 2600 pmoles/min/mg after 72 h. Transport rates declined later on while the cultures reached confluency. The spontaneous increase was strongly inhibited by dexamethasone (60%) in marked contrast to the 5 to 7-fold induction of this transport system in hepatocytes [1].
In spite of these differences concerning regulation inhibitor studies could not clearly distinguish the Na^+-dependent transport system in BEC from system G^- in hepatocytes.
Our results demonstrate that BEC have a strong capacity for uptake of glutamate which may be involved in the resorption of glutamate in bile liberated from glutathion conjugates by the action of γ-glutamyl-transferase. The inverse regulation by dexamethasone distinguishes this transport from that in hepatocytes and may be of regulatory importance.

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Klonierung, Sequenzierung und Überexpression von HPr aus S. carnosus

HPr und EnzymI sind die zuckerunspezifischen, cytoplasmatischen und konstitutiv expremierten Proteinkomponenten des bakteriellen Phosphotransferase-Systems. Das für das HPr kodierende ptsH-Gen und das für das EnzymI kodierende ptsI Gen sind sowohl in E. coli als auch in B. subtilis im pts-Operon organisiert (1;2).

Zur Klonierung des ptsH-Gens wurde S. carnosus DNA mit HindIII vollständig restringiert, die DNA-Bruckstücke in den Expressionsvektor pUC19 inseriert. Diese Genbank wurde in E. coli JM109 transformiert. Zur Identifizierung S. carnosus HPr expremierender Klone wurden ca. 6000 Einzelkolonien mittels eines Immuntests untersucht. In drei mit dem polyklonalen anti-S. carnosus HPr Antiserum reagierenden Klonen konnte in einem in vitro Komplementationsversuch HPr-Aktivität nachgewiesen werden. Daraufhin wurden Subklonierungsversuche des 3.5 kB großen DNA-Fragmentes durchgeführt und schließlich ein 800 bp großes DNA-Fragment sequenziert. Auf dem sequenzierten DNA-Fragment befindet sichh das komplette ptsH-Gen und die ersten 300 Nucleotide des ptsI-Gens. Für Überexpressionsversuche wurde ein 600 bp-großes DNA-Fragment in den Vektor pT7-5 kloniert und in den E. coli Stamm K38 pGP1-2 transformiert. Aus 10 g Bakterienzellen konnten insgesamt 125 mg S. carnosus HPr isoliert werden. Das auf diese Weise gewonnene Protein ist hochrein, EnzymI-abhängig und HPr-Kinase abhängig phosphorylierbar und kann auch für 2D-NMR Spektroskopie verwendet werden.

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Charakterisierung von E. coli HPr-Mutanten

Das HPr (heat stable protein) des bakteriellen Phosphotransferase-Systems wird als Phosphogruppendistributor EnzymI-abhängig phosphoryliert und überträgt die Phosphogruppe auf verschiedene zuckerspezifische EnzymIII/EnzymII Komplexe (1). Das aktive Zentrum dieses nur 10 kD großen Proteins wird durch H15, R17 und E85 ausgebildet (2).
Um die Beteiligung verschiedener Aminosäuren des HPr bei der Interaktion mit anderen Proteinen zu untersuchen, wurden durch ortspezifische Mutagenese die HPr- Mutanten PE11, EA68 und 85 erzeugt. Die Proteine wurden überexpremiert und bis zur Homogenität gereinigt. HPr PE11 und EA68 sind im Gegensatz zum Wildtyp HPr durch $EnzymI_{S. carnosus}$ schneller phosphorylierbar als durch $EnzymI_{E. coli}$. HPr 85 ist ebenfalls EnzymI-abhängig phosphorylierbar. Des weiteren wurden die Phosphotransfereigenschaften dieser Mutantem im Glc-PTS und im Man-PTS getestet. Während HPr EA68 eine dem WT-HPr identische Übertragungsrate aufweist, ist der Phosphogruppentransfer von HPr PE11 und HPr 85 um ca. 40% herabgesetzt.
Zur näheren Untersuchung einer leaky HPr- Mutante mit dem Phänotyp $Gut^- Mtl^{(+)} Nag^{(+)}$ wurde mittels PCR das für das

HPr kodierende ptsH-Gen aus dem E. coli Stamm JLT3 amplifiziert, in den Vektor pUC18 kloniert und sequenziert (3). Das HPr aus diesem HB101-Derivat zeigt einen Aminosäureaustausch PS18 und gibt somit einen Hinweis auf eine verminderte Interaktion mit dem EnzymIII^{gut}.

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The role of subunit δ of chloroplast F_0F_1 ATPase in ATP synthesis

Membrane-bound CF_0CF_1 ATPase catalyses ATP synthesis during photophosphorylation at the expense of a transmembrane protonmotive force. The enzyme consists of a membrane-embedded proton-conducting part, CF_0, and an extrinsic, nucleotide binding portion, CF_1. The latter consists of five different subunits with a stoichiometry of $(\alpha\beta)_3\gamma\delta\epsilon$.

The large subunits α and β are responsible for substrate turnover and some of the smaller subunits γ, δ and ϵ are believed to be responsible for coupling proton flow to ATP synthesis. We investigated the role of subunit δ. EDTA treatment of thylakoids removes CF_1 and opens the proton channel CF_0. We found that δ can remain on CF_0 keeping it closed [1]. Further evidence for the ability of δ to 'plug' open CF_0 came from the demonstration that addition of a $\beta_3\delta$ complex and isolated δ could restore photophosphorylation in partially CF_1-depleted thylakoids [2,3]: In view of the lack of any catalytic activity of isolated δ this could be explained only by the assumption that δ plugged leaks through open CF_0 thereby allowing for membrane energization.

It also could be demonstrated that *E. coli* δ partially fulfilled the role of chloroplast δ in photophosphorylation because it improved the reconstitution of photophosphorylation by $CF_1(-\delta)$ [4]. Therefore it is likely that subunit δ fulfills the same function in both chloroplast and *E. coli* F_0F_1 ATPase.

Since subunit δ, which is located between F_0 and F_1, controls proton flow, it might participate either directly in proton conduction, funneling protons from the channel into the active site, or it might act as a conformational transducer. For several reasons discussed we favor the second role.

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A cation binding site of bacteriorhodopsin

Bacteriorhodopsin (bR) the only protein of the purple membrane can be converted into a blue membrane by deionization. Several authors proposed cation binding sites which were assumed to be responsible for the stabilization of the purple colour and which may be of functional importance.
In this study a cation binding site of bR comprising the loop between helices C and D and the anquor of the C-terminal tail could be identified by sequence analysis of peptides modified with a cobalt III pentaammine complex (1). This result is substantiated by Solid State NMR measurements of (4-^{13}C)-Asp labeled bR which revealed that the removal of the C-terminus exposes Asp residues to the aqueous phase (2).
The modification of the protein by the Co reagent is enhanced by activation of the photocycle. This indicates that this part of the membrane surface alters its structure during the transport of the proton and seems to be involved either functionally or spatially in the capture of the proton from the cytoplasma.

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C_4-Dicarboxylate Transport in Rhizobium meliloti

Symbiotic nitrogen fixation is an energy demanding process. In the rhizobium - legume symbiosis the supply of the microsymbiont with sufficient carbon sources is an important step. C4-dicarboxylates are thought to be the energy source essential for nitrogen fixation in the bacteroid. The C_4-dicarboxylate transport system of the Rhizobia plays a central role in starting and maintaining nitrogen fixation.

We isolated several Tn*5* induced *R. meliloti* mutants lacking the C_4-dicarboxylate tranport system in the free living state. These mutants were divided into three classes according to their different phenotypes and different genetic loci. The Tn*5* insertions of classI mutants were localized in the structural *dct*A gene, those of classII mutants map in or near the regulatory *dct*B gene and Tn*5* insertions of classIII mutants map in the *rpoN* (*ntr*A) gene. The *dctA* and *dct*B genes are neighbored but transcribed in opposite directions in *R. meliloti*. Homologies to the arrangement of the *dct* genes in *R. leguminosarum* indicate the presence of a second regulatory gene, *dct*D, downstream of the *dct*B gene.

The *dctA* gene is preceeded by a "nif"-consensus promoter sequence probably interacting with RpoN. Additionally, an upstream element was found where NifA may bind to. In the transcriptional regulation of the dctA gene the regulatory gene products DctB and DctD also play a role which is still not clear.

Interpreting the sequencing data of *dctA* and *dctB* we suggest that DctA is the C_4-dicarboxylate transport protein localized in the *R. meliloti* membrane and the DctB is a sensor protein which is less strong associated to the membrane.

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PAS1, a Gene Essential for Peroxisome Biogenesis

Recently, we described a screening procedure for the isolation of peroxisomal mutants of S. cerevisiae[1]. Among the resulting mutants two groups could be distinguished. One group (pas mutants) is characterized by the absence of morphologically detectable peroxisomes and mislocalization of peroxisomal matrix enzymes in the cytosol. These properties strongly suggest a defect in peroxisome assembly which may be due to impaired protein sorting. Thus, pas mutants should potentially be useful to characterize essential components of the peroxisomal import machinery.

As a first step to dissect the mechanism of peroxisome biogenesis we have undertaken the molecular cloning and analysis of the PAS1 gene. Functional complementation of the pas1 mutant by a genomic library led to a DNA fragment which after subcloning and sequencing revealed a large open reading frame predicting a protein sequence of 1043 amino acids. No significant sequence similarities extending over the entire length of the ORF exist between the PAS1 gene and sequences im the PIR protein or GeneBank DNA databases, neither at the nucleotide nor the amino acid level. However, a specific region of about 150 amino acids shows a remarkable degree of similarity with regions of the two proteins SEC18 and NSF. These have been reported to be required for fusion processes associated with protein secretion and to be functional equivalents in S. cerevisiae and CHO cells, respectively[2]. This region which is conserved in all three proteins may represent a functional domain necessary for a fundamental step in protein sorting.

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Mannose Permease of Escherichia coli: Domain Structure and Function of the Phosphorylating Subunit

The mannose permease of Escherichia coli transports mannose and related hexoses by a mechanism that couples sugar transport with sugar phosphorylation. It consists of two transmembrane subunits (II-P^{Man} and II-M^{Man}) and a hydrophilic subunit (III^{Man}). III^{Man} also exists in a soluble dimeric form in the cytoplasm. The III^{Man} subunit consists of two structurally and functionally distinct domains which are linked by a flexible hinge of the sequence KAAPAPAAAAPKAAPTP AKP. Both domains are transiently phosphorylated. The N-terminal domain (P13) is phosphorylated at N-3 of His-10 by the cytoplasmic phosphorylcarrier protein phospho-HPr. The C-terminal domain (P20) is phosphorylated by P13 at N-1 of His-175. Phosphoryltransfer occurs not only between P13 and P20 on the same III^{Man} subunit but also between isolated domains and between domains on different subunits of the dimer. In the presence of the II^{Man} subunits, the phosphorylgroup is directly transferred from His-175 of P20 to the sugar substrates of the permease.

The P13 domain contains the contact sites for dimerization of III^{Man}. The P20 domain contains the contact sites for interaction with the II^{Man} subunits. By reconstructing the ptsL gene, the two domains were expressed as individual polypeptides and the length of the hinge between P13 and P20 was changed. The in vivo and in vitro activities of mutant III^{Man} were little affected by these modifications.

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Changes in gene expression and protein phosphorylation in plants in relation to fungal elicitors as biotic signals

Fungal elicitors effect gene expression in plant cells in a very specific way. Among the early responses of plant cells are the de novo synthesis of enzymes responsible for the formation of phytoalexins, i. e. stilbenes in the case of peanut and grapevine cells. Although the bulk of glycoproteins secreted into the extracellular compartment is not rapidly changed upon elicitor treatment, a few distinct components of the system responsible for the assembly of the cell wall are selectively enhanced within 2 h of elicitation. The very early part of the signal transduction process originating from the biotic elicitor can be found at the plasma membrane. These processes on the cell surface may be studied in vitro by testing for receptors or by assaying the changes in the qualitative and quantitative pattern of protein phosphorylation. We isolated and purified plasma membranes and nuclei from cultured peanut cells, and assayed for internal protein kinase activities. Some of the protein de novo syntheses observed upon cell fractionation are separate phenomenon unrelated to elicitation by fungal wall fragments but the synthesis of a 52 kDa protein was observed only when nuclei isolated from induced cell were analyzed. Besides the non-phosphorylated 52 kDa nuclear protein which was specifically induced 4 h after elicitor treatment we detected phosphoproteins with 88 kDa, 66 kDa, 48 kDa, and 22 kDa in the isolated nuclei. The phosphorylation of a 37 kDa nuclear protein was stimulated by spermine. In plasma membranes, proteins with 150 kDa and 58 kDa were highly phosphorylated, while proteins exhibiting a size of 48 kDa, 44 kDa and 56 kDa varied in phosphorylation depending on time.

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Cyclic AMP Binding Protein and Phosphodiesterases in Volvox carteri

Sexuality is initiated in Volvox carteri by a 28 kDa glycoprotein at 10^{-16} M. The external signal has to be transduced into the gonidia by an internal second messenger chain. In order to elucidate the impact of cAMP-signal transduction, we isolated - among other elements of such a cascade - a cAMP binding protein and two cAMP dependent phosphodiesterases by ion-exchange and gel-filtration chromatography. The cAMP-binding protein (38kDa) was purified to electrophoretic homogeneity by affinity chromatography on cAMP agarose. Its dissociation constant K_d is 200 nM as determined by Scatchard plot.

^{3}H-cAMP binding was assayed by a precipitation technique. Phosphodiesterase was measured by radio immuno assay using a monoclonal antibody (1) or HPLC-reversed-phase-chromatography (1). Cyclic AMP binding activity elutes in two peaks (70 mM and 150 mM NaCl) on ion exchange chromatography. When either one of the two peaks is subjected to gel-filtration chromatography, the binding activity is found again in two fractions. The first contains proteins of 60 to 100 kDa. The second peak is much retarded, thus its molecular mass cannot be estimated with any accuracy. As also sucrose density centrifugation of crude extract yields two peaks of 70 kDa and 35 kDa, it is concluded that the cAMP binding protein is present in in the cell in a monomeric and an aggregated form. The aggregate is either a dimer or a complex of the cAMP binding with another protein.

Phosphodiesterase elutes at 110mM NaCl on ion-exchange chromatography; it has a molecular mass of 40 kDa (gel-filtration). The sole reaction product is 5' AMP; nevertheless kinetic analysis points to two different PDE activities. K_m of the less specific enzyme is 166,6 µM, V_{max} 16,6 pmoles/minute. In contrast, K_m and V_{max} of the more specific PDE were 1,1µM and 4,2 pmoles/minute respectively. Only the more specific enzyme is inhibited by IBMX.

Both Volvox enzymes show specifities similar to those of mammalian systems; thus a cAMP regulated signal transduction is probable in this flagellate. At present it is investigated whether the binding protein is the regulatory subunit of a cAMP dependent kinase.

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Properties of the Locust Vitellogenin Receptor

In many egg-laying animals, such as insects, yolk protein precursors, the vitellogenins, are extraovarially synthesized, released into the haemolymph and transported to the ovaries. Maturing oocytes sequester vitellogenins by receptor-mediated endocytosis. This process includes the binding of vitellogenin to a receptor molecule associated with the oocyte cell membrane and the subsequent internalization of the receptor-ligand complex embedded in coated vesicles. From African migratory locusts, (Locusta migratoria), the vitellogenin receptor has been identified and purified by affinity chromatography. The receptor was extracted from oocyte membranes by solubilization with the nonionic detergent octyl-ß-D-glucopyranoside retaining its high affinity binding properties (K_d = 4.2 x 10^{-8} M) (1). After removal of the detergent the receptor was subjected to affinity chromatography on vitellogenin coupled covalently to Affigel 15. The binding protein was eluted with a buffer containing EDTA and Suramin at low pH. It was identified and visualized by ligand blotting on a nitrocellulose membrane (with PAP method) as a protein with an apparent molecular weight of 156 kDa by sodium dodecylsulphate polyacrylamide gel electrophoresis under non-reducing conditions (2). The isoelectric point of the protein, which is in the range of pH 3.6, was determined by isoelectric focussing in the presence of the detergent. In combination of western blotting with glycan detection the receptor could be identified as a glycoprotein. The specific binding of vitellogenin increases from 4.8 µg (intact oocyte membranes) to 170.9 µg (affinity purified receptor) per mg membrane protein which corresponds to a purification factor of 35.

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Site-directed mutagenesis on the lacF-gene of S.aureus

$EIII^{lac}$, a sugar specific component of the staphylococcal phosphotransferase-system, catalyzes the transfer of a phosphoryl-group from the cytoplasmatic protein HPr to an integral membrane enzyme EII^{lac} which is specific for lactose substrate.

Digestion with trypsin and Glu (C)protease of the ^{32}P-labelled $EIII^{lac}$ followed by peptide separation and analysis revealed that His 82 is the PEP-dependent phosphorylation site (1).

The nucleic acid sequence of $EIII^{lac}$ was published by Breidt et al. (2) and confirmed the AA-sequence.

A great degree of AA-sequence homology was found for the enzymes III^{lac} from S.aureus, S.lactis (3) and L.casei (4). Comparison of those sequences reveals that His 82 of $EIII^{lac}$ in L.casei is replaced by a Thr residue, therefore His 78 seems to be responsible for the only weak enzyme activity.

Nevertheless, it seems unlikely to find His 78 as the active center of $EIII^{lac}$ from S.aureus and S.lactis, because both of them possess a His 82 and show a strong transfer of their phosphoryl-groups to their EII^{lac}.

A plasmid carrying the isolated lacF-gene from S.aureus was subjected to do site-directed mutagenesis in order to exchange His 82 to Ser 82 and gain further information whether His 82 is part of the active center or not.

E.coli strain JM83 containing either the lacF-wildetype or mutant gene respectively were tested in the mutant complementation assay (5). The mutants show only weak activity compared with the wildetype.

During isolation of $EIII^{lac}$-Ser82 we observed a different behaviour compared to the wildetype protein.

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Biochemical and Molecular Characterisation of the Mannitol-specific Enzyme III from Staphylococcus carnosus

We report the purification of enzyme III^{mt1} from S.carnosus, the characterisation of the active center, cloning and sequencing of the gene, and similarity to the mannitol-specific enzyme II from E.coli.

After phosphorylation of purified Enzyme III^{mt1} with (^{32}P)PEP, enzyme I and HPr, the protein was cleaved with endoproteinase Glu(C). The aminoacid sequence of the peptide carrying the phosphoryl-group was found to be Ala-Val-Val-Ser-Thr-Phe-Met-Gly-Asn-Gly-Leu-Ala-Ile-Pro-His-Gly-Thr-Asp-Asp. We assume the single histidyl residue to carry the phosphoryl group, since all proteins of the PTS, which have been investigated so far, indeed carry the phosphoryl group attached to a histidyl residue.

Genomic libraries of S.carnosus DNA were constructed using the expression vector pUC19 and $EIII^{mt1}$ producing clones were identified using rabbit poly-

clonal antiserum. A 700-bp DdeI fragment containing the complete gene encoding EIII^{Mtl} was sequenced by the dideoxy chain-termination technique. Upstream from the ORF for EIII^{Mtl} one can find a sequence analogous to that of the E.coli promoter. This region acts as a strong promoter when subcloned into the promoter test vector M13HDL17. EIII^{Mtl} was overproduced using an inducible T7 polymerase system and purified to homogeneity. Amino acid sequence comparison confirmed a 38% similarity to the hydrophilic enzyme III like portion of enzyme II^{Mtl} of E.coli.

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Partial Purification and Characterization of Acid Phospholipase A_1 from Rat Liver Golgi Vesicles

Acid phospholipase A_1 (PLA1) activity has been shown to be an intrinsic constituent of the rat liver Golgi system [1]. Activity characteristics closely resemble those of the corresponding lysosomal enzyme. We have therefore presumed [1,2] a biogenetic relation between Golgi PLA1 and lysosomal PLA1. The present studies have been performed to further characterise the Golgi PLA1.

Golgi vesicles were isolated from rat liver by isopycnic centrifugation (3). The purity of the preparation was assessed biochemically by determining several marker enzymes, and morphologically by electron microscopy. As determined by the Golgi marker galactosyl transferase, Golgi vesicles were purified about 139-fold, with a yield of 4 - 6 %. Approximately 70 % of the PLA1 activity was solubilised. It was subsequently purified about 70-fold with a yield of 29 - 51 % over the Golgi vesicle fraction, by using a micromodification of the affinity column chromatographies in series developed previously for the purification of rat liver lysosomal PLA1 [4,5].

The final preparation displayed a specific catalytic activity of about 42 mU/mg protein towards 200 µM sonicated phosphatidylethanolamine at pH 4.5. Upon chromatofocusing by FPLC, the PLA1 activity eluted as a single homogeneous peak at pH 5.3. However, the purified Golgi PLA1 activity in native isoelectric focusing displayed isoelectric points of 5.7 (major form), 5.3 and 4.7, respectively. Native gradient polyacrylamide slab gel electrophoresis revealed PLA1 activities at molecular mass ranges of 58 (major form), 38 and very little of 17 kDa, respectively. SDS polyacrylamide slab gel electrophoresis of purified rat Golgi PLA1 revealed only molecular masses of 58 (major form) and 38 kDa.

The molecular weights and isoelectric points observed for the isoenzymes of Golgi PLA1 are only slightly different from those detected for lysosomal PLA1 from rat liver [4,5]. Our results further support the assumption [1] that acid Golgi PLA1 isoenzymes are the catalytically active precursors of the corresponding lysosomal PLA1.

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Expression and intracellular localization of CBS1, a translational activator of mitochondrial cytochrome *b* in yeast

Translation of yeast mitochondrial cytochrome *b* RNA specifically depends on two nuclear genes, *CBS1* and *CBS2*, which encode highly basic proteins of molecular weight 27 and 45 kDa, respectively[1,2].

In vitro import studies show that the 27 kDa CBS1 protein is the precursor form of a soluble mitochondrial 23,5 kDa protein[1]. The mitochondrial targeting sequences reside in the aminoterminal end of the precursor protein: while truncation in the carboxyterminal end does not affect import, deletion of aminoterminal sequences abolishes import into isolated mitochondria. Interestingly, when under the control of the strong *ADC1*-promotor and at the same time present on a high copy number plasmid, the aminoterminally truncated form of CBS1 is able to functionally complement *cbs1*-mutants. We interprete this result in terms of a bypass import into mitochondria.

The amino acid sequence of CBS1 shows a motif which is reminescent of a sequence commonly found in RNA-binding proteins. Preliminary UV-crosslinking studies hint at the possibility that CBS1 can interact with the cytochrome *b* RNA-leader. This unusual long RNA-leader has previously been identified by genetic means as the target site of CBS1 (as well as of CBS2)[3].

Expression of *CBS1* varies in response to oxygen and the carbon source[4]: the concentration of the *CBS1* transcript is significantly higher in aerobically and glucose-derepressed grown cells as compared with anaerobically

and glucose-repressed cultured cells. A qualitative similar result is obtained by monitoring the ß-galactosidase activity of a *CBS1/lacZ*-fusion construct under various growth conditions. However, a quantitative evaluation shows that the observed differences in ß-galactosidase activity are smaller than those seen in the *CBS1*-transcript concentration. We are currently testing, whether a small mini-open reading frame in the 101 b long 5'-leader of the *CBS1* transcript mediates this effect.

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Multidrug Resistant Human Leukemia Cell Lines Exhibiting Amplification and an Inducible Expression of a P-Glycoprotein Gene

Several members of the mdr gene family (mdr = multiple drug resistance) have been characterized so far from different species. They code for membrane anchored P-glycoproteins (140-170 kD) which most likely act as energy dependent efflux pumps for several groups of lipophilic, but structurally and functionally unrelated cytotoxic compounds. Using mdr1 and mdr3 specific cDNA fragments we analysed the gene copy number and the corresponding mRNA expression of these genes in three highly cross-resistant CCRF-CEM cell lines selected in vitro with actinomycin D, vincristine or adriamycin, respectively. In either case the amplification (3- to 12-fold) of the mdr1 and the mdr3 gene could be demonstrated, only the mdr1 mRNA was expressed, though. Recently, we reported the actinomycin D induced mdr1 expression in an actinomycin D selected CCRF subline (1). We demonstrate here that either actinomycin D or adriamycin induce within 72 h a 2- to 3-fold increase of the mdr1 mRNA steady state level in two multidrug resistant CCRF sublines which were selected with actinomycin D or vincristine, respectively. In further experiments we recorded the actinomycin D and adriamycin dose response curves by evaluation of [^{3}H]uridine or [^{3}H]thymidine incorporation into the acid insoluble material. Consistently, the drug sensitivity of the respective macromolecular synthesis was found to decrease with increasing mdr1 mRNA levels. In case of an adriamycin selected multidrug resistant subline, however, a similar mdr1 inducibility could not be detected so far. Moreover, during the cultivation of the actinomycin D selected subline over several months in the presence of actinomycin D the inducibility of the P-glycoprotein gene expression was lost almost completely.

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Binding and Lateral Diffusion of a Signal Peptide in Model Membranes

The synthetic 25 residue mitochondrial signal peptide of cytochrome oxidase subunit IV (coxIV-25) [1,2] was labeled with the fluorophor N-((2-(iodoacetoxy)ethyl)-N-methyl)amino-7-nitrobenz-2-oxa-1,3-diazole (IANBD) at its single cysteine residue at position 19. This derivative (NBD-coxIV-25) was purified by reversed phase HPLC. Binding of NBD-coxIV-25 to small unilamellar phospholipid vesicles was measured by the increase of the fluorescence intensity upon its incorporation into the hydrophobic environment of a membrane. Vesicles of different composition of POPC (zwitterionic) and POPG (negatively charged) were used. The relative fluorescence intensity increase was markedly enhanced by the presence of POPG. In addition to the observed hydrophobic interactions, this effect indicated strong electrostatic interactions of the positively charged peptide with negatively charged vesicles.
Lateral diffusion of the peptide in multilamellar planar phospholipid bilayers was measured by fluorescence recovery after photobleaching. Again, the lateral mobilities of the peptide depended on the charge on the model membrane. For instance, the peptide diffused with a lateral diffusion coefficient of $2.8*10^{-8}$ cm^2/s in bilayers composed of POPC and POPG (80:20) and with $8.1*10^{-8}$ cm^2/s in bilayers of pure POPC at 21° and at a peptide- to-lipid mol ratio of 1:1900. For a comparison, the lateral diffusion coefficient of the fluorescent lipid probe NBD-eggPE was $5.2*10^{-8}$ cm^2/s, irrespective of the lipid composition. Our binding and lateral diffusion results are consistent with the notion of a deeper penetration of the peptide into the hydrophobic core of the lipid bilayer in the presence of negatively charged lipids. The requirements of negatively charged lipids for an efficient insertion into membranes may be physiologically relevant for mitochondrial protein import.

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Use of peptides for raising antibodies against the ribosomal protein S6 and for studying its phosphorylation

Growth-promoting signals (growth factors, insulin) cause the rapid phosphorylation of the ribosomal protein S6 of higher eukaryotes at several C-terminal seryl residues. S6 phosphorylation seems to aid in factor-induced activation of protein synthesis. S6 is, however, also a target for signals (e.g. glucagon, NGF) known to activate the cAMP signalling pathway. We have embarked on studies on S6 and on S6-phosphorylating kinases. A human mitogen-responsive S6 kinase has been found[1], and the cDNA for human S6 has been sequenced. The 18 amino acid C-terminus (RRLSSLRASTSKSESSQK, identical for man[2], mouse, rat) has a Ser in position 15, where a Glu was anticipated based on earlier protein sequencing work.

Using the earlier sequence data, 1) antisera were raised against C-terminal epitopes and 2) synthetic peptides were offered as *in vitro* substrates to S6-phosphorylating protein kinases. Ad 1: Cross-reaction of certain polyclonal antisera with S6 homologues from non-mammalian sources (chicken, yeast and - as a preliminary observation - *Artemia salina*) was observed. - As a different application, in immunofluorescence studies, the antibodies caused strong staining of nucleoli of cultured cells. Ad 2: The following enzymes were able to phosphorylate peptides representing S6 sequences: cAMP-dependent protein kinase, mitogen-responsive S6 kinase, protein kinase C. The former two kinases prefer different sites both on intact S6 and on the peptide RRLSSLRA. Theoretically, these site specificities at the S6 level could relate to antagonism of different signalling pathways (controlled e.g. by glucagon or insulin).

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The ATP-Synthase of Chloroplasts: Kinetic Investigations of the Mechanism of the Enzyme

The ATP-synthase from chloroplasts, CF_oF_1, was isolated and reconstituted into asolectin liposomes. ATP hydrolysis was measured with these proteoliposomes and with thylakoids under conditions where only one binding site is occupied ("single site"). The substrate and enzyme concentration is in the nM range so that single steps of the reaction cycle can be measured ("single turnover conditions"). One catalytic site alone is able to make a complete reaction cycle. The rate constants were measured for the ATP binding ($1*10^6$ $M^{-1}s^{-1}$), ADP release (0.1 s^{-1}), P_i release (0.2 s^{-1}) and the equilibrium between ATP and ADP/P_i bound on the enzyme (0.4). The interaction of the different nucleotide binding sites was investigated by the transition to high substrate concentrations (1 mM ATP). The ATP, which was bound on the first site, was hydrolyzed under these conditions with a rate of 0.5 s^{-1}; whereas, under the same conditions, ATP on the other sites was hydrolyzed with a rate of 80 s^{-1}. Therefore, the enzyme has two different types of catalytic sites which work independently.

On thylakoids it is possible to make these measurements also under energized conditions. The energization leads to an increase of the affinity for ADP (and a decrease for ATP) to the enzyme in the range of more than a factor 1000. Therefore, it can be shown that the protonation/deprotonation steps are involved in the release of ATP and the binding of ADP and P_i.

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Human Preprocathepsin H: cDNA Sequence and Expression in E. coli

The lysosomal cysteine proteinases cathepsin B, H, and L are thought to play a major role in intracellular protein turnover and possible involvements in extracellular processes like inflammation, tumor invasion and hormone activation are discussed [1]. In contrast to other thiol proteinases cathepsin H (EC 3.4.22.16) has endo- and aminopeptidase activity [2].

Several cDNA clones from libraries of human kidney and monocyte RNA were isolated and characterized [3]. Sequence data show that human cathepsin H is synthesized as a preproprotein of 335 residues with a molecular mass of 38000. Similarity with the rat enzyme is very high (>80%) even in the prepro region.

Strategies for heterologous expression of the mature enzyme in E. coli were investigated. Direct cytoplasmic expression did not yield detectable gene products despite of demonstrable transcription. Fusion to the signal sequence of the periplasmic alkaline phosphatase of E. coli leads to low-level production of two inactive specific polypeptides. Instead of the expected translocation to the periplasm apparent transport inhibition was observed resulting in cytotoxic effects.

High-level synthesis was achieved by fusing cathepsin H to the protein A of S. aureus, additionally allowing purification by IgG-affinity chromatography. The fusion protein precipitated as inclusion bodies that were readily solubilized by urea. For recovery of the cathepsin H moiety a factor Xa cleavage site was included in the fusion protein.

Proper refolding conditions are now going to be established. Site-directed mutagenesis should then allow detailed analysis of the relevance of distinct residues for catalysis and specificity of this cysteine proteinase and for inhibitor interactions.

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Insertion of actin monomers between the barbed ends of actin filaments and barbed end-bound insertin, a protein purified from smooth muscle

An actin polymerization-retarding protein was isolated from chicken gizzard smooth muscle. This protein copurified with vinculin on DEAE cellulose and gel filtration columns. The polymerization-retarding protein could be separated from vinculin by hydroxylapatite chromatography. The isolated polymerization-retarding protein lost its activity within a few days, but was stable for weeks when it was not separated from vinculin. We termed the polymerization-retarding protein "insertin". Because of the instability of the isolated insertin we investigated the effect of insertin-vinculin on actin polymerization. Insertin-vinculin retarded nucleated actin polymerization maximally five-fold. Polymerization at the pointed ends of gelsolin-capped actin filaments was not affected by insertin-vinculin, suggesting that insertin-vinculin binds to the barbed ends, but not to the pointed ends of actin filaments. Retarded polymerization was observed even if the actin monomer concentration was between the critical Concentration of the two ends of treadmilling actin filaments. As at this low monomer concentration the pointed ends depolymerize, monomers appeared to be inserted at the barbed ends between the terminal subunits and barbed end-bound insertin molecules. Insertin may be an essential part of the machinery of molecules which permit treadmilling of actin filaments in living cells by insertion of actin molecules between membranes and actin filaments.

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IP_3-induced calcium release from isolated vacuoles of *Neurospora crassa*

In animal cells, inositol 1,4,5-trisphosphate (IP_3) is reported to release calcium ions from endoplasmic reticulum. In contrast, the fungus *Neurospora crassa* possesses vacuoles, which are able to sequester Ca^{2+} and release it after stimulation by IP_3. The calcium release was demonstrated by applying two independent methods, measuring free calcium ions through complexation with the fluorescence indicator fura-2, and loading the vacuoles with ^{45}Ca and determing the retained radioactivity. Calcium is released from isolated vacuoles upon heat shock without further stimulation. At an experimental temperature of 25°C, however, the leakage is rather small and efflux is induced by a faktor of more than 20 by addition of IP_3, showing a striking dose-dependence with a K_m of 5.28 μM. Furthermore, it could be demonstrated, that dantrolene, characterized before as a blocker of calcium channels in sarcoplamic reticulum, inhibits the IP_3-induced Ca^{2+} release from *Neurospora crassa* vacuoles almost quantitatively.

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Hormonal Adaptation of Amino Acid Transport in Response to Liver Injury by CCl_4

Exposure of rat liver to CCl_4 is well known to cause severe necrosis in zone 3 of the hepatic acini and, thus, may considerably influence amino acid uptake by the liver due to the heterogeneity of amino acid transport capacities across the acinus, e.g. predominance of Na^+-dependent glutamate uptake in zone 3 (1). In the present study we have focussed on changes of amino acid uptake and its hormonal regulation in cultured hepatocytes isolated at various times after exposure of rats to CCl_4 (1ml/kg; injections i.p. on 3 consecutive days).

Hepatocytes were isolated at various times after cessation of CCl_4 exposure, cultured for 48 h in the absence or presence of different hormones and the Na^+-dependent uptake of α-aminoisobutyric acid (AIB), histidine (HIS) and glutamate (GLU) was determined as described (1,2). Results obtained 3-5 days or 12-21 days after CCl_4-intoxication were pooled for early (E-reg) or late (L-reg) regeneration, respectively.

AIB transport in control cultures was little affected, while its induction by dexamethasone (D) and glucagon (G) increased more than 2-fold after CCl_4-damage. HIS uptake dropped to 60% during E-reg and increased during L-reg in controls and after induction with D and insulin (I). GLU uptake was unchanged in controls, whereas the induction by D was repressed after CCl_4-damage and markedly increased during L-reg. The presence of D/I strongly suppressed the induction during L-reg.

These results demonstrate pronounced acute alterations in amino acid transport due to CCl_4-intoxication corresponding to the predominant acinar localization of the different amino acid transport systems. Furthermore, they reveal a complex hormonal regulation of the various transport agencies in association with the altered hormonal status during different phases of the regenerating process.

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Phospholipase induced changes of Mammalian Cell Electrokinetics and their Correlation to Sensitivity against Pseudomonal Cytotoxin

A cytotoxin from *Pseudomonas aeruginosa* damages mammalian cells by forming pores in plasma membranes resulting in increased permeability for small molecules. Sphingomyelinase pretreatment of cells increases the cell sensitivity to cytotoxin

whereas Phospholipase D pretreatment is without effect. It is not clear, whether charges of phospholipids on cell surface may influence the attack of cytotoxin.
Since the charges influence the electrophoretic movement, the movement in Free Flow Electrophoresis can indicate whether the surface charge is changed by phospholipase pretreatment. Free Flow Electrophoresis was carried out in an electric field of 100 V/cm and an exposure time of 2 min in a K/Na phosphate buffer containing NaCl, glucose and sucrose with a conductivity of 2,1 mS/cm.
Pretreatment of Ehrlich mouse ascites cells and rabbit erythrocytes by Sphingomyelinase (EC 3.1.4.12) or/and Phospholipase D (EC 3.1.4.4) increased the cell electrophoretic mobility towards the anode in a similar extend, indicating that positive charges were splitted off enzymatically. We conclude that cytotoxin toxicity on the tested cells may not be influenced mainly by surface charges.
In controls, electrophoresis of cells after Neuraminidase (EC 3.2.1.18) pretreatment resulted in mobility decrease. However, the effect of all three enzymes was small. It indicates that the surface potential depends not only on the number of charged molecules at the surface but also on their position to the surface of shear.

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Localization of the UGA Dependent Translational Termination Domain in 16S rRNA

The catalytic function by rRNA in the initial steps of the translational cycle is by now well documented. Here we report on 16S RNA sequence motifs that participate in UGA dependent translational termination (1). A number of 16S rRNA mutants, constructed by site directed mutagenesis, were screened for their ability to suppress all three termination codons. This was achieved *in vivo* utilizing a system in which ribosomes must translate through a stop codon in order to synthesize functional ß-Galactosidase. Based on the paradigm of structure-function correlation the results can be interpreted in terms of two molecular interactions: firstly, base pairing between the UGA stop codon in mRNAs and helix 34 in 16S RNA; secondly, base pairing between the region encompassing base 726 and helix 34. Moreover, these interactions provide a role for release factor 2. Therefore this would now complete the evidence, that rRNA directly participates during all stages of protein biosynthesis (2).

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Synthesis and Degradation of Hyaluronic Acid by Synovial Macrophages

We found that phagocytosing cells on the surface of synovial membranes (SM) contained ten times more specific Hyaluronate acid (HA) - synthase activity than all other cells. HA is responsible for viscosity in joints of normal patients and has a lower weight-average molecular weight in the joint with rheumatoid arthritis (RA) (1). The SM contains four different cell types: macrophages, fibroblasts, dendritic cells and lymphocytes (2). These were solubilized by collagenase treatment. Phagocytosing cells were feed with magnetic beads and retained by a magnetic field. The most likely mechanism by which HA could be depolymerized in an inflammed joint involves oxygen-derived free radicals, notably superoxide anion (3). In comparison to arthrosis synovial cells we observed production of superoxide anion after Phorbol-Myristate-Acetate (PMA) stimulation in a higher rate by all RA synovial cells .
Macrophage dependent synthesis and degradation of HA may therefore be associated in the same microenvironment.

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P. Gräber

The ATP-Synthase from Chloroplasts

The ATP-synthase from chloroplasts is a membrane bound enzyme which catalyzes H^+-transport coupled ATP synthesis/hydrolysis. The enzyme contains nine different subunits. The hydrophilic CF_1 part (five subunits) is connected to the membrane-integrated CF_o part (four subunits) by a small stalk. The enzyme can be isolated and reconstituted into liposomes with activities similar to that of chloroplasts. The ATP-synthase can exist in at least four different states: oxidized, reduced and active/inactive. These different states have different catalytic properties. The rate of ATP synthesis/hydrolysis has been measured after a $\Delta pH/\Delta\Psi$ jump with rapid mixing techniques as a function of

ΔpH, $\Delta\Psi$ and substrate concentrations with the different forms of the enzyme. Investigation of ATP hydrolysis at low substrate concentrations ("single site", "single turnover" conditions) shows that there are at least two catalytic sites on the enzyme with different properties.

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Solubilization and Functional Reconstitution of the Plasmalemma Ca^{2+}-ATPase from *Commelina communis* L.

The plasmalemma Ca^{2+}-ATPase from leaves of *C.communis* is a well characterized enzyme (Gräf and Weiler,Physiol.Plant.(1989) in press) involved in pumping Ca^{2+} ions out of the cell and thus maintaining low free levels of cytoplasmic Ca^{2+}. As a prerequisite of a detailed molecular study of the properties, regulation and structure of this enzyme, its solubilization and reconstitution in functional form are necessary. The Ca^{2+}-ATPase was solubilized from highly enriched plasma membrane vesicles prepared from leaf microsomes by aqueous two-phase partitioning in dextran T500/polyethyleneglycol 3350 phase systems. Conditions for the optimum solubilization were worked out using ATP-driven Ca^{2+}-transport into reconstituted proteoliposomes as a functional assay. An efficient solubilization/reconstitution system was set up and this serves in the purification of the functional Ca^{2+}-ATPase.

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Basolateral expression of chicken liver glycoprotein receptor and rat liver asialoglycoprotein receptor in transfected MDCK cells

We have studied the targeting and function of two basolateral receptors from chicken and rat hepatocytes after transfection of their cDNAs into the polarized cell line MDCK (Madin-Darby canine kidney).
The chicken glycoprotein receptor, a single subunit receptor, is expressed at the basolateral plasma membrane of MDCK cells, where it performs binding, uptake and degradation of ligand.
The rat liver asialoglycoprotein receptor consists of two subunits, designated RHL-1 and RHL-2/3. When expressed alone, newly synthesized RHL-1 is rapidly degraded intracellularly with a half-life of 2 h. RHL-2/3 is much more stable (half-life > 12 h) and is targeted to the basolateral plasma membrane. In cells expressing both subunits, RHL-2/3 rescues RHL-1 from rapid degradation, which results in the expression of a functional receptor at the basolateral membrane of MDCK cells. These studies indicate that sorting signals for targeting and endocytosis and the cellular machinery decoding them are conserved between different species and cell types. They open the way for the analysis of the molecular mechanisms involved in the sorting and recycling of basolateral epithelial receptors.

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Effects of Conditioned Media from Normal and Regenerating Hepatocytes on Proliferation and Proteoglycan Synthesis of Rat Liver Fat Storing Cells in Culture

Fat storing cells (FSC, ITO cells) are located in the immediate proximity of parenchymal liver cells (PC) in the space of Disse. Beside their role in storage and metabolism of retinoids FSC proliferate, transform into (myo-)fibroblasts and secrete large amounts of extracellular matrix components (collagens, proteoglycans) in injured liver sections and, thus, contribute significantly to tissue repair and fibrosis. A role of Kupffer cells in the activation of FSC has been established recently, but additional paracrine effects of neighbouring PC on the proliferation status and proteoglycan (PG) synthesis/secretion have not been established so far.
Methods: PC were isolated (90 % trypan blue negative, 85 % single cells) by the collagenase perfusion technique from normal and regenerating liver 1 d, 3 d, 4 d, and 5 d after 2/3 hepatectomy (Hep X). The cells were seeded at a density of 10^6/75 cm^2, and kept for 26 h in culture in Dulbecco's modified Eagle's medium with 0.5 % and 10 % fetal calf serum (FCS), respectively. Conditioned medium was collected during 24 h, the cell free supernatant was added at various dilutions to monolayers of fat storing cells ($0.4 \cdot 10^6$/10 cm^2) kept in medium with 0.5 % ("proliferation assay") and 10 % ("inhibition assay") FCS, respectively. The proliferation of FSC

was assayed by measuring [^{3}H] thymidine incorporation and DNA-content per well, respectively. Synthesis and secretion of PG were determined by incorporation of [^{35}S] sulfate into medium glycosaminoglycans, which were specified by nitrous acid chondroitin AC-, ABC-lyase treatment.

Results: Medium from regenerating PC 4 d and 5 d after Hep X stimulated [^{3}H] thymidine incorporation into FSC kept in 0.5 % FCS in a dose-dependent manner. Maximum stimulation of 87 % was reached at a 1 : 2 dilution of conditioned medium from PC 4 d after Hep X. Similarly, the DNA content per well increased also. Conditioned medium from normal PC and PC 1 d after Hep X did not affect significantly DNA content and [^{3}H] thymidine incorporation rate of FSC. Medium from normal PC in 10 % FCS added to proliferating FSC in 10 % FCS did affect neither the DNA content nor [^{3}H] thymidine incorporation ruling out any soluble proliferation inhibitor secreted by PC. The synthesis of medium PG by FSC (kBq/mg DNA) was enhanced by a maximum of about 45 % if conditioned media from regenerating and normal PC were added to FSC in 0.5 % FCS. This effect was mainly due to a strong increase of chondroitin sulfate accompanied by a relative decrease of dermatan sulfate. Heparan sulfate remained essentially unchanged.

Conclusion: (i) Conditioned medium from regenerating PC (4 and 5 d after Hep X) but not from normal PC stimulates the proliferation of FSC possibly due to TGF-alpha, (ii) PC do not secrete an inhibitor of FSC proliferation, (iii) both normal and regenerating PC secrete a factor stimulating production and secretion of proteoglycans, in particular of chondroitin sulfate, in FSC.

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Soluble Glycoproteins of VSV are Transported in a Trimerized Form

VSV infected BHK-21 cells release soluble derivates of the G-protein into the grwoth medium which are recognized by polyclonal antibodies raised against VSV G protein. These G_s proteins lack the cytoplasmic domain of the authentic membrane-integrated G protein[1], are translated on polysomes bound on the rough endoplasmic reticulum (rER) and glycosylated cotranslationally[2]. Modifications and folding are important processes during or shortly after translation giving rise to G protein trimers which are transported from rER via the Golgi to the cell surface. The kinetics of carbohydrate modification from high-mannose to complexoligosaccharide side-chains and the intracellular transport of VSV glycoproteins can be followed by using of endoglycosidase H which does not remove complex carbohydrates that are produced in the late Golgi compartment. Pulse/chase experiments demonstrate that the G protein and the soluble derivates become endo H resistent 20 min. after synhthesis.G protein of VSV New Jersey is detectable on the cell surface 30 min. after translation on the rER. The extent of G protein trimerization was determined by sucrose gradient centrifugation at pH 5.8 at which trimeric G protein remain stable whereas at neutral pH the trimers tend to dissociate into monomers. Gradient fractions containing trimers were immunoprecipitated with antisera which were raised against the cytoplasmic domain of the G protein and analyzed by SDS-PAGE. Two trimeric forms of G protein differing in molecular weight were detected after gel electrophoresis under nonreducing conditions.

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I. Grotjohann and P. Gräber

Isolation of the CF_o Part from the ATP-Synthase from Chloroplasts

The ATP-synthase from chloroplasts is built from a hydrophilic part, CF_1, and a hydrophobic, membrane-integrated part, CF_o. It is assumed that protons are translocated through the CF_o part when the enzyme catalyzes ATP synthesis/hydrolysis. In order to facilitate investigations of the mechanism of proton transport, the CF_o part has been isolated as follows: CF_oF_1 was isolated and purified as usual. Then, CF_oF_1 was reconstituted into asolectin liposomes. Finally, the CF_1 part was removed by NaBr treatment. SDS-gel electrophoresis shows the CF_o part is composed of subunits I,II,III and IV. The reconstituted CF_o shows a proton transport of 4 $H^+/(CF_o \cdot s)$ when the liposomes are energized by a K^+/valinomycin diffusion potential.

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The "ΔpH"-Probe 9-Aminoacridine: Methodological Queries and the Photophysical Mechanism of Fluorescence Quenching

The fluorescent probe 9-aminoacridine (9-AA) and its derivatives became one of the most popular tools for quantitative determination of transmembrane pH gradients (inside acidic) and of their decay kinetics. We have tested the function of 9-AA in a variety of pure lipid and reconstituted protein-lipid vesicles in order to elucidate the underlying molecular processes involved in ΔpH sensing and fluorescence quenching. Several of the results obtained, e.g., the occurrence of fluorescence changes in the absence of a pH-gradient upon energization of membrane systems, the absence of fluorescence quenching in the presence of large pH-gradients, the strict requirement of net-negatively charged lipids for the occurrence of quenching and the cooperative dependence of the quenching extent on the number of negative charges, and the missing kinetic correlation between ΔpH decay and fluorescence recovery, are incompatible with the previously proposed reaction mechanism and with the applicability of 9-AA as a reliable, quantitative reporter of transmembrane ΔpH[1]. From the analysis of 9-AA's optical spectra and its fluorescence lifetimes, a photophysical

reaction mechanism of the ΔpH-induced fluorescence quenching could be inferred, which is based on the formation of excimers at the membrane surface resulting from excited ground state dimers[2].

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A.H. Guse, I. Berg and G. Gercken

Metabolism of inositol phosphates in homogenized cardiac myocytes

Previous studies demonstrated the accumulation of multiple inositol phosphate (InsP$_x$) isomers in agonist-stimulated cultured cardiac myocytes of adult rats [1].

To confirm the pathways of Ins(1,4,5)P$_3$ metabolism, homogenates of isolated myocytes were incubated in separate experiments with [^{3}H]Ins(1,4,5)P$_3$ or with its two direct products [^{3}H]Ins(1,3,4,5)P$_4$ and [^{3}H]Ins(1,4)P$_2$ for different periods and the metabolites were analysed by anion-exchange- and by NH_2-HPLC.

The dephosphorylation of tritium-labelled Ins(1,3,4,5)P$_4$ started rapidly within the first 3 min and was accompanied by a concomitant increase in Ins(1,3,4)P$_3$. Between 3 min and 15 min Ins(1,3,4,5)P$_4$ and Ins(1,3,4)P$_3$ decreased at a constant rate, while Ins(3,4)P$_2$, Ins(1,3)P$_2$ and InsP$_1$ increased. Ins(3,4)P$_2$ was the major InsP$_2$ isomer.

When [^{3}H]Ins(1,4,5)P$_3$ was added to the homogenate it was metabolized in two ways. The main pathway was the dephosphorylation to Ins(1,4)P$_2$. The minor pathway was the phosphorylation to Ins(1,3,4,5)P$_4$ and the catabolism to Ins(3,4)P$_2$ and Ins(1,3)P$_2$.

In a separate experiment Ins(1,4)P$_2$ was shown to be degraded exclusively to Ins(4)P$_1$, whereas Ins(1,3,4,5)P$_4$ via Ins(1,3,4)P$_3$, Ins(3,4)P$_2$ and Ins(1,3)P$_2$ yielded only Ins(1)/(3)P$_1$.

The data presented here suggest a metabolism of Ins(1,4,5)P$_3$ via direct dephosphorylation as well as via phosphorylation to Ins(1,3,4,5)P$_4$ and subsequent dephosphorylation as described for other cells.

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Light-Induced Lateral Diffusion of Plastocyanin in Chloroplast Thylakoids

The electron transport complexes of higher plants are heterogeneously distributed with photosystem (PS) II in appressed grana, PS I in non-appressed and cytochrome (cyt) b_6/f complex in both membrane regions [1]. Several questions related to the long-range electron transport between the two membrane regions and the function of cyt b_6/f complexes in appressed regions are not fully understood. In addition to plastoquinone, plastocyanin may be sufficiently mobile to shuttle fast enough across the long distances. Its lateral distribution in the thylakoid lumen has been probed in thin-sections of leaves from spinach and pea with mono-specific antibodies and visualized by binding of protein A-gold [2]. The electron micrographs indicate a twofold concentration in the stromal as compared to the granal lumen in the dark. Laser flash-induced fast reduction of $P700^+$ by complexed plastocyanin indicates that in stroma regions most of plastocyanin is bound to PS I and mobile plastocyanin is preferentially localized in grana regions. The fraction of plastocyanin in the grana lumen increases at the expense of that in the stroma region by a factor of two during illumination. This is evidence for a light-driven diffusion of plastocyanin form stroma to grana regions. The result suggests that cyt b_6/f in grana may be more efficiently connected to linear electron transport by plastocyanin in the light than in the dark.

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J. Hafer, B. Brockhage, A. Siebers and E.P. Bakker

The Three Subunit Kdp K^+-Uptake ATPase Complex from the Thermoacidophilic Bacterium *Bacillus acidocaldarius*

Like *E. coli* the thermoacidophile *B. acidocaldarius* expresses a high-affinity K^+-translocating Kdp-ATPase when grown at low K^+ concentrations in the medium. The isolated enzyme strongly resembles the *E. coli* Kdp system in that i) it consists of three different subunits; ii) it has a very high affinity for K^+ ($K_m \leq 10$ μM); iii) its activity is stimulated by the same monovalent and divalent cations as is the *E. coli* enzyme. The two large subunits of the enzyme from the acidophile cross-react with antibodies generated against the *E. coli* KdpB-protein (catalytic subunit) or KdpA-protein (function in K^+ binding), suggesting that the two Kdp-ATPases have a very similar mode of action. Like other P-type ATPases the *B.*

acidocaldarius enzyme can be phosphorylated by ATP. However, the extent of phosphorylation of the enzyme prepared with the non-ionic, non-dialyzable detergent Aminoxid WS35 was with 3% very low. Experiments are in progress aimed at isolating the enzyme in a more native form with the aid of a detergent that will enable subsequent reconstitution of the Kdp-ATPase in an artificial phospholipid membrane.

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Search for Control Elements in the S6 Gene Responsible for Coordinate Ribosomal Protein Expression

The primary coordinate control of ribosomal gene expression which is responsible for the production of equimolar amounts of ribosomal proteins (rp) occurs at the level of transcription. Additional fine-tuning mechanisms operate at the posttranscriptional and/or translational level. Conserved sequence boxes in the rp-gene promoter and in the 5'non-coding regions are possible control elements. Using hybridisation-techniques we isolated a cDNA clone (S6-5) of human ribosomal S6 protein which contains the complete coding sequence and 25 bp of the 5'noncoding region. This clone was sequenced in order to resolve the differences in the published human S6 cDNA sequences[1,2]. Furthermore a specific S6 clone has been isolated from a dog cDNA library. It consists of 93 bp of the 5'noncoding region and 128 bp of the amino terminal coding region. When compared to the human S6 cDNA a difference of 12 nucleotides became apparent, three of them give rise to a conserved amino acid exchange. Calculations show that the sequences of the 5'noncoding regions allow the formation of stable secondary structures ($G=\Delta$-7,2 to -11,2 kcal/mol) which might affect the translational efficiency. In order to learn more about control elements in the S6 gene which might be involved in the coordinate expression we have started to analyse human S6 genomic clones. Preliminary restriction analyses indicate that one clone contains intervening sequences whereas the other clones most likely represent pseudogenes.

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Polyol Dehydrogenases from Gluconobacter oxydans: Purification and Characterization of a Cytoplasmic Glucose Dehydrogenase

During our investigation of the carbohydrate metabolism of gluconobacter we isolated and characterized three different sorbitol dehydrogenases (SDH I-III) from cells grown in a sorbitol-containing medium. The purification procedure of these enzymes yielded also one glucose dehydrogenase (Glc DH). In respect of the evolution of the polyol dehydrogenases we have also focussed our interest on this enzyme.

Glucose dehydrogenase was isolated by fractionated ammonium sulfate precipitation, chromatography on DEAE-cellulose and pseudo-affinity chromatography on Fractogel-TSK-Blue. By changing the elution conditions, we could now isolate both enzymes SDH I and Glc DH from the Fractogel TSK-Blue column. Each enzyme was obtained in an apparent homogeneity and a good yield.

Glucose dehydrogenase catalyses the oxidation of D-glucose and D-mannose using only NADP as coenzyme. The app. K_m-values are 8 mM for the hexoses and 0.1 µM for the coenzyme. In contrast to the glucose dehydrogenases from B. megaterium or subtilis, the enzyme is inactive with NAD. The optimum pH value for glucose and mannose oxidation is in the range of 8.5 - 9.5. The enzyme is rather stable at pH values of 8.0 - 10 and at elevated temperatures (up to 65 °C). The enzyme is not inhibited by sulfhydryl reagents. The molecular mass of the subunit was determined to be 40 000 and the native enzyme is probably composed of two identical subunits. The amino acid composition and amino terminal sequences were determined. From the sequence homology to Glc DH from B. megaterium it may be derived that the coenzyme binding domain is located in the amino terminal region.

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A. Hamann, S. Kluttig, E.P. Bakker

The constitutive K^+ - uptake system Trk from *Escherichia coli*

In *Escherichia coli* are at least three K^+ uptake systems involved in the K^+ accumulation in the cytoplasm. The Trk system is expressed constitutively and is responsible for potassium uptake under most growth conditions. The activity of the Trk system is regulated by the cell turgor and requires both a high cytoplasmatic ATP concentration and high transmembrane proton motive force. The Trk system seems to be composed of several proteins encoded by genes (*trkA, trkE, trkG, trkH*) scattered on the *Escherichia coli* chromosome .

Mutations in the *trkA* gene abolish the Trk activity, whereas mutations in one of the other *trk* genes have not this drastic effect on K^+ accumulation. The copy number of the Trk proteins in the cell is very low (10-30 copies). Sequencing data on *trkA* showed, that the regulation of the copy number of TrkA is based on the transcriptional and translational level. The promoter sequence and Shine-Dalgarno box possess only a low homology with procaryotic consensus sequences, whereas a consensus liked terminator is formed by a stem out of 12 basepairs. The TrkA protein was identified as a peripheral membrane protein. The other gene products seemed to be involved in anchoring the TrkA to the cytoplasmic membrane. The preliminary sequence of the *trkG* show that its low expression is obviously based on the transcriptional level, it showed up a typical promoter region but a very weak Shine- Dalgarno sequence. One open reading frame and biochemical studies indicate that TrkG is an integral membrane protein.

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Th.Hamann and M.A.Müller

Fluorescence-microscopic and Immunocytochemical Localization of Catecholamines in an Annelid

The catecholaminergic nervous system of the proterandric hermaphrodite Ophryotrocha puerilis is examined by glyoxylic acid-induced fluorescence (GIF) staining method[1] and PAP-immunocytochemistry with an polyclonal antibody against dopamine (DA)[2].

20 - 30 neurons of the brain containing catecholamine (CA) have been observed in the prostomium by GIF. Most of those cells are well stained by immunocytochemistry. Fluorescent i.e. DAimmunoreactive nerve fibres and tracts have been found in the neuropil.
Konnectives of the ventral nerve cord are passed through by paired GIF and DAimmunoreactive axons. Some neurons of the ventral nerve cord ganglion projected fluorescent and DAimmunoreactive fibres to the parapods, where also some marked neurons are located.

The descriped CAergic nervous system might have a senso-motoric function. A possible influence of DA to sexual development of O. puerilis had been discussed elsewhere[3].

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The Membrane-bound and the Intracellular Forms of the Ki-1 Antigen both Possess Protein Kinase Activity

The Hodgkin-associated Ki-1 antigen consists of a 120 kD membrane-bound glycoprotein and a 57 kD intracellular non-glycosylated form. They are synthesized independently of each other. Both are phosphorylated at serine residues.
An analysis of the peptide fragments resulting from staphylococcal V8-protease digestion revealed identical bands for the 57 kD molecule irrespective of the cell source. Some bands of the 57 kD-digests also appeared among the peptide fragments of the 120 kD membrane antigen.
Both forms of the Ki-1 antigen exhibited protein kinase activity. The 120 kD, but not the 57 kD molecule, was immunoprecipitated from cell lysates of Hodgkin-analogous cell lines L428 or L540, which had been loaded with the Ki-1 antibody (method 1). The 57 kD Ki-1 antigen, devoid of the 120 kD form, was isolated from L540 cells after removal of the membrane form by method 1 or from U266/B1 myeloma or Raji and Daudi Burkitt lymphoma cells which only contain the smaller form of the Ki-1 antigen. Effects of non-specific adsorption were ruled out by various control precipitates. - It was observed that both the membrane-associated 120 kD and the 57 kD intracellular form of the Ki-1 antigen showed auto-phosphorylation and could phosphorylate other substrates as well.

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Comparative Studies on Pyruvatekinase from PSE- and Normal Muscles

Pyruvatekinase (EC 2.7.1.40) was isolated from Musculus longissimus dorsi of pigs with normal and PSE (pale, soft, exudative) character. PSE-pork is of minor sensorical quality which is formed after slaughter by a 5-8 times faster breakdown of glycogen ending with lactic acid than in muscles of normal behaviour. The pure enzymes were compared with regard to their structural and catalytic properties[1].

Homogeneous pyruvatekinase from PSE-muscles showed in contrast to the same enzyme from normal muscles a higher specific activity. According to the K_{cat}/K_m-relation the enzyme isolated from PSE-meat is almost ten times more active than the pyruvatekinase from meat of normal character. The increased catalytic effectiveness depends essentially on a lower K_m-value for the substrate phosphoenolpyruvate, whereas in the case of the second substrate adenosine-5'-diphosphate no differences in the K_{cat}/K_m-value could be determined. Significant differences between both enzyme species were detected in the pH-dependance of the reaction.

During the isolation procedure both enzymes showed an identical behaviour. A molecular weight of 52000 ± 2000 for the subunits of both enzymes was determined by dissociating gelelectrophoresis.

In the case of pyruvatekinase from normal meat two main bands were identified by isoelectric focussing techniques, whereas for the enzyme of PSE-meat a distinct third band appeared.

The different enzymatic properties of pyruvatekinase of PSE- and normal muscles were not due to differences in the degree of phosphorylation.

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Kinetics of the Trypsinogen Activation by Enterokinase and Trypsin

Trypsinogen is secreted into the duodenum by pancreatic cells. The activation of trypsinogen to trypsin is accomplished by the proteolytic enzyme enterokinase and is the first step of a cascade of events. The released trypsin catalyzes the activation of trypsinogen in an autocatalytic process (1,2).

A global kinetic analysis of the mechanisms of the trypsinogen activation by enterokinase and trypsin is developed. The kinetic equations of both the transient phase and steady state are presented for these mechanisms. In addition we here derive the corresponding kinetic equations for the case in which the condition of rapid equilibrium prevails. Since in vivo enterokinase and trypsin coexist, we have obtained the kinetic equations assuming that at the onset of the reaction, both enterokinase and trypsin are present together with the trypsinogen and a substrate of the trypsin. So,the physiological conditions are optimally simulated.

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J. Heberle and N.A. Dencher

Time-Resolved Proton-Movement Across Bacteriorhodopsin Monitored by an Optical pH-Indicator Bound to the Membrane Surface

Bacteriorhodopsin (bR), the light energized proton-pumping protein in the purple membrane (PM) of *H. halobium*, was covalently labeled with the pH-indicator FITC (Fluorescein-5-isothiocyanat). Fluorescence pH-titrations were carried out and compared with the bulk pH-indicator pyranine (8-Hydroxy-1,3,5-pyrene-trisulfonate). The variation of the KCl-content shows that the surface-pH strongly depends on the charge density of the protein environment, e.g. the pH is lowered by 1.5 pH-units at low salt concentration. The same investigation was performed with FPE (N-(5-Fluoresceinthiocarbamoyl) dipalmitoyl-L-α-phosphatidylethanolamine) doped reconstituted bR/DMPC-vesicles.
If monitored with FITC, the appearance and reuptake of the actively pumped protons at the bR-surface could be resolved in time and directly correlated with the formation and decay of the photocycle intermediate M. However, if the appearance of the proton in the aqueous bulk phase is measured with pyranine, a delay by a factor of 5 is observed. This indicates a rate limiting step in the proton diffusion from membrane surface to the bulk water phase[1]. Even acceleration of the H^+-movement by means of mobile buffer addition could not match the rise of the pyranine signal with the rise of the M-intermediate.
Our experiments demonstrate that the kinetics of proton-movement in the microsecond time range can only be accurately determined with surface bound indicators. This finding is of relevance not only for the elucidation of bR's H^+-pumping mechanism but has to be considered for all H^+-delivering and consuming membrane proteins.

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J.H. Hegemann, G. Cottarel and R.Stoll

DNA Requirements for Centromere Function in S.cerevisiae

The stable maintenance of genetic information during meiosis and mitosis depends upon highly accurate mechanisms of chromosome segregation. In the yeast, Saccharomyces cerevisiae, missegregation of a chromosome is an extremely rare event, occuring only once in every 100.000 mitotic cell divisions and once in every 10.000 meiotic cell divisions. Of essential importance to this high fidelity of chromosome transmission is the centromere region of the chromosome. The centromere (CEN) provides the site of attachment to the mitotic and meiotic spindles. In S.cerevisiae, the centromere DNA of 12 of the 16 chromosomes has been cloned. They contain within 111 to 119 bp 3 conserved DNA elements: CDEI, CDEII, and CDEIII. Recently we could show, that a 125 bp CEN6 DNA fragment comprising only these conserved elements is sufficient for mitotic and meiotic centromere function (1).

A detailed analysis of the DNA sequence requirements for proper centromere function is a prerequisite to understand the processes involved in centromere assembly and centromere action. We therefore decided to determine the contribution of individual base pairs of CDEI and CDEIII to centromere function by a detailed mutational analysis. Oligonucleotide directed mutagenesis was used to introduce specific base changes. Their effects on centromere activity were quantified using the chromosome fragment assay. Up to now we characterized 14 base pair changes in CDEI which increase chromosome loss rates 3 to 20 fold compared to wild type. The so far analysed 20 point mutations in CDEIII result in 2 to 100 fold increased loss rates and in some cases in a complete loss of centromere function.

Furthermore we started to analyse selected centromere mutants with the in vivo footprint technique. Comparison of the footprint pattern obtained for mutants with the pattern for wild type CEN6 allows us to correlate the DNA - protein footprint and mitotic centromere function. We are presently extending this correlation by characterizing selected CEN mutants for their meiotic behavior.

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Inhibition of Proteinkinase C by Tiflucarbine

Multiple lines of evidence indicate a close relationship between the physiological pathways regulated by Ca^{2+}/calmodulin (CaM) and Ca^{2+}/phospholipid dependent proteinkinase C (PKC). Accordingly, several drugs such as sphingosine or some phenothiazines are known to inhibit both CaM and PKC activities. In the present study, we therefore investigated whether the putative antidepressant tiflucarbine (BAY P 4495), which has previously been shown to bind to calmodulin (CaM) and to inhibit CaM-dependent cyclic nucleotide phosphodiesterase, is also able to inhibit PKC-mediated reactions in vitro and in vivo. Tests performed include histone phosphorylation by partially purified PKC from rat brain, zymosan (ZyC3b) or phorbol ester induced generation of reactive oxygen species from cultured human polymorphonuclear leukocytes determined by lucigenin-amplified chemiluminiscence and cell proliferation of a human skin keratinocyte cell line (HaCaT), estimated by means of ^{3}H-thymidine or ^{14}C-amino acid incorporation. In all three test systems, tiflucarbine exhibited a dose-dependent inhibition with similar IC_{50}-values in the low micromolar range. It is thus concluded that tiflucarbine, in addition to its CaM-antagonistic effects, also effectively inhibits PKC-dependent processes in vitro and in situ.

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Comparison of ATP-driven with ADP-stimulated Calcium and Anion Transport in Sarcoplasmic Reticulum

Active transport ot Ca^{2+}, driven by ATP hydrolysis, can be accompanied by the accumulation of either, oxalate or phosphate, at a stoichiometric ratio of one [1]. Thus, the capacity of the vesicles for calcium oxalate/calcium phosphate uptake can be increased by exceeding their solubility products. It is generally accepted that anions passively follow Ca^{2+} through a seperate anion channel.
However, calcium and anion accumulation can also be shown by stimulation with Mg-ADP. Thus, calcium and phosphate are transported in parallel in the absence of an energy donor (and presence of an adenylate kinase inhibitor) but presence of either a calcium or a phosphate gradient. Varying either gradient results in an identical increase of both, calcium and phosphate uptake, and leaves the 1:1 stoichiometry untouched. Calcium and phosphate transport in dependence on the calcium as well as on the phosphate concentration show positive cooperativity with comparable maximal velocities and Hill coefficients.
This data indicates that calcium and anions might be co-transported across the SR membrane. We speculate that this co-transport is accomplished by the Ca^{2+}-ATPase [Ec 3.6.1.38].

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In vitro Import of Peroxisomal Membrane and Matrix Polypeptides

The import system used in the present studies was obtained from a rat liver derived cell-free in vitro translational system (1) which consists of a postmitochondrial supernatant containing the microsomal and the cytoplasmic fraction of the liver homogenate. For protein synthesis this homologous system utilizes its endogenous mRNAs (2). Studies on the distribution of the newly synthesized peroxisomal proteins revealed that some portions were confined to the particulate fraction. Thus, the microsomal fraction contains organelles, most likely microperoxisomes, which were highly competent in importing peroxisomal proteins.
Two integral peroxisomal membrane proteins (PMP69 and PMP22) became inserted into the membrane in this system in a carbonate resistant manner (pH 11). Moreover, treatment of the membranes with proteases did not affect the integrity of PMP22 and led to a characteristic fragmentation of PMP69 exactly as observed with the in vivo inserted polypeptides (2).
The matrix enzyme 3-ketoacyl-CoA thiolase (EC 2.3.1.9) which is known to be synthesized with a 2 kDa N-terminal extension was cleaved at the import to its mature size. The imported mature enzyme which was protease resistant could only be digested following the addition of detergents.
The import of another matrix enzyme acyl-CoA oxidase (EC 1.3.3.-) which, different to thiolase, contains the carboxy-terminal tripeptide targeting signal -Ser-Lys-Leu, could also be demonstrated by protease treatment in the absence and presence of detergents.
Thiolase import was not affected by the presence of 10-50 uM of various monovalent cationic ionophores e.g. valinomycine, nigericine, monensin and carbonylcyanide m-chlorophenylhydrazone suggesting that import of peroxisomal thiolase does not require an active membrane potential.

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Expression and Genomic Organization of Catenin, an Intermediate Associated Protein

By using the biological system of chicken chondrocytes in culture we were able to construct

cDNA clones from sternal mRNA and select for those which belong to RNA species decreasing in amount during prolonged culture. For one of these clones (p550) the corresponding gene was isolated and partially sequenced. The transcription of this gene results in two RNA species of 4.4 and 6.5 kb in length depending on two different polyadenylation sites. With RNAse H experiments it was possible to estimate the relative positions of the cDNA clone. The exact site of the transcription start has been determinated by S1 Nuclease analysis and a primer extension experiment.
Northern blots of different embryonal tissues demonstrate a developmental dependence of the transcription. We characterized the genomic organization and analyzed almost the whole cDNA sequence.
The deduced amino-acid sequence of the cDNA revealed internal repeats in Catenin with considerable homology to the domains of a yeast protein. Southern blots of genomic DNA of different species (yeast, chicken, human) reveal the presence of this gene in a broad range of eucaryotes.
Immunofluorescence studies showed a close codistribution of Catenin with the intermediate filament system.

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Isolation of a trifunctional crosslinking amino acid (c.a.a.) from skin and arterial tissue and its characterization by mass- and high resolution NMR- spectroscopy

After enzymatic digestion of the unsoluble collagen a trimeric crosslinking peptide was obtained releasing after acid hydrolysis a component the nominal mass of which was shown to be 444 Da applying FAB MS. From the exact masses of the adducts $(M+H)^+$=445.241 Da and $(M+Na)^+$=467.223 Da, as measured by TOF-SIMS, the elemental composition of the substance was concluded to be $C_{18}H_{32}O_7N_6$.
From mass spectroscopic data the hypothesis can be established, that the substance (M^+=444) is a trifunctional c.a.a. that is formed by addition of histidin (M^+=155) to the unsaturated dimeric aldimine c.a.a. dehydro-hydroxylysinorleucine (M^+=289) consisting of a lysine and hydroxylysine component.
A 500 MHZ ^{1}H-NMR spectrum and Selective Homonuclear Double Resonance Techniques demonstrated 3 groups of protons, which mainly could be assigned to the protons of lysine, hydroxylysine and histidine.
As shown by a proton broadband decoupled ^{13}C spectrum 7 methylene, 5 methine, 3 heteroaromatic and 3 carboxyl carbons are found. The occurrence of 3 α-methine and 3 carboxyl carbons underline the trifunctional character of the c.a.a.
The presence of a ^{1}H-singlet in the aromatic region and two quaternary carbons in the imidazolyl ring indicate that histidin is added to the -C=N- double bond of the precursor dimeric c.a.a. via the imidazolyl-proton His H-2'.

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S. pombe - a new host for the functional expression of integral membrane proteins

Recently it has been shown that the archaebacterial light-driven proton pump, bacteriorhodopsin (BR) from *Halobacterium halobium*, can be expressed in the fission yeast *Schizosaccharomyces pombe* [1].
The functional expression of BR in the eukaryotic cell is remarkably different to the expression of modified bacterioopsin (BO) genes in *E. coli*:
The expression of BO is harmful for *E. coli* [2], but not for *S. pombe*. The transformed yeast cells show no reduction in growth, as was reported for *E. coli*.
BO is very unstable in *E. coli*. It is degradated in 8-10 min. In fission yeast BO is stable for more than two days.
The consitutive expression of the authentic bop-gene, encoding for the precursor, and the expression of the sequence encoding for the mature protein is possible in yeast without modification of the coding region, but is impossible in the eubacterium.
The natural leader sequence of Pre-BO is processed in yeast by cleavage with the leaderpetidase to mature BO. Proteolytic cleavage of BO fusion proteins in *E. coli* has never been reported, indicating a wrong folding of the fusion proteins. BO integrates in yeast membranes, but is aggregated in the cytosol of *E. coli* when no homologous leadersequence was used.
In vivo regeneration of the pigment is achieved in *S. pombe* by addition of retinal as in *H. halobium*, but not in *E. coli*. BO expressed in the eubacterium has to be isolated and reconstituted in artificial liposomes.

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Identification of the Plastocyanin Binding Subunit of Photosystem I

The subunit of higher plant photosystem I (PS I) which is assumed to be necessary for an efficient electron transfer from plastocyanin to P700 has been termed Subunit III of PS I [1]. But its attribution to a particular band after SDS-PAGE of PS I is ambiguous in subsequent reports. We have cross-linked plastocyanin specifically to a subunit of PS I with N-ethyl-3-[3-(dimethylamino)propyl]carbodiimide (EDC) similar as described [2] by incubation of stroma lamellae and isolated PS I. Analysis of the peptide composition shows that this subunit with an apparent molecular mass of 18.5 kDa disappears and a new band at 31 kDa appears which contains cross-linked plastocyanin as recognized by anti-plastocyanin antibodies. The N-terminal amino acid sequence of the subunit is identical to that of the mature peptide coded by the *psa*F gene which contains an excess of positively charged residues [3]. Except for one amino-acid residue it is identical to the initial sequence of a PS I subunit in pea which had been suggested to function in ferredoxin binding [4]. Plastocyanin cross-linked to PS I shows reduction kinetics of $P700^+$ with a halftime of 13 μs which is characteristic for the native complex between plastocyanin and PS I. We conclude that a carboxyl group of the negatively charged plastocyanin is cross-linked to a lysine residue of subunit III in the orientation of the native complex.

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Complete Primary Structures of Porcine α_1-Microglobulin and the Inhibitor Subunit of Porcine Inter-α-Trypsin Inhibitor

The amino-acid sequences of α_1-microglobulin and the inhibitor subunit of inter-α-trypsin inhibitor (ITI) could be deduced from cloned porcine liver cDNA. As the respective human proteins, porcine α_1-microglobulin and the inhibitor subunit of porcine ITI result from proteolytic processing of a common primary translation product.

The physiological functions of these serum proteins are not yet clear. Structural data suggest that α_1-microglobulin is a member of a transport protein family, called the α_{2u}-globulin family. But up to now, only the human sequence was known completely [1]. Comparison of the amino-acid sequences of α_1-microglobulins of several species may help to identify functionally important structural features of these proteins.

The inhibitor subunit of porcine ITI belongs to the well-studied group of Kunitz-type proteinase inhibitors. At least its human homologue is discussed also as an endothelial cell growth factor [2]. Obvious deviations of the deduced amino-acid sequence from that observed earlier [3] will be discussed.

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Competitive Inhibition of Liver and Brain Catechol-O-Methyltransferase by Tetrahydroisoquinoline Alkaloids

Tetrahydroisoquinoline alkaloids e.g. salsolinol (1-methyl-6,7-dihydroxytetrahydroisoquinoline), tetrahydropapaveroline (norlaudanosoline, 1-(3',4'-dihydroxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline) [1] and norlaudanosoline-1-carboxylic acid [2] can be formed in mammals by condensation of dopamine and aldehydes or 2-oxo-acids. The first step of a possible pathway in the metabolism of these compounds is an O-methylation catalyzed by S-adenosyl-L-methionine: catechol-O-methyltransferase (EC 2.1.1.6), an enzyme which is also of importance in the metabolism of catecholamines.

It is known that tetrahydroisoquinolines are not only able to influence the transmitter function but also the metabolism of catecholamines. For example, it was shown by incubation experiments using partially purified catechol-O-methyltransferase from various tissues that certain tetrahydroisoquinolines competitively inhibit the methylation of various catecholic compounds provided that they are themselves substrates for the enzyme [3,4].

Our incubation experiments using the catecholamine metabolite 3,4-dihydroxybenzoic acid as substrate and S-adenosyl-L-[methyl-^{14}C]methion-

ine as methyl donor[5] revealed that the methylation of the substrate by catechol-O-methyltransferase enriched from rat liver[6] was competitively inhibited by salsolidine (1-methyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline) (Ki = 0.19 mM) and 1-carboxysalsoline (1-carboxy-1-methyl-6-hydroxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline (Ki = 0.44 mM), which are themselves not substrates for the enzyme. Furthermore, by using an enzyme assay with catechol (o-dihydroxybenzene) as substrate in the presence of S-adenosyl-L-[methyl-^{3}H]methionine[7] we found that salsolidine competitively inhibits the methylation of this substrate by the soluble and the membrane bound catechol-O-methyltransferase enriched from pig brain according to a method described by Nissinen[8]. The inhibitor constants were found to be Ki = 7.4 mM and Ki = 5.3 mM respectively indicating that the membrane bound form is inhibited to a higher extent than the soluble form of the brain enzyme.

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Urokinase in breast tumor cells: Immunohistochemical localization and quantitation by ELISA

The urokinase-type plasminogen activator is produced by human tumor cells as an inactive single-chain form. Pro-uPA can be transformed into the enzymatically active form (uPA) by small amounts of plasmin. uPA then acts on plasminogen to form plasmin which degrades the tumor stroma (fibrin-fibronectin matrix) and thus facilitates tumor progression and metastasis.

Degradation of tumor pro-uPA by other proteases such as neutrophil elastase and thrombin leads to an enzymatically inactive form of uPA whereas treatment with trypsin or plasmin generates active uPA. The uPA chains caused by elastase, thrombin, plasmin, or trypsin treatment are linked by disulfide bonds and do not differ in apparent molecular weight as judged by SDS-PAGE.

Pro-uPA and also protease-treated pro-uPA were recognized by monoclonal antibodies (moAB) #394 and #377 (American Diagnostica, New York, USA). With these moAB a highly sensitive ELISA was set up (detection limit 40 pg uPA / ml) which was used to quantitate pro-uPA and uPA in urine, plasma, and tumor tissue extracts. Breast tumor tissues (n = 115) were extracted with 1 % Triton-X-100 and the content of uPA assessed. On the average 3.1 ng urokinase / mg protein was measured compared to benign controls (n = 30; 0.23 ng urokinase / mg protein).

Urokinase was located in the cytoplasma of tumor cells in breast cancer tissues by moAB #394 (APAAP method). In the same tumor sections neutrophils were stained by an antibody to neutrophil elastase supporting the notion that degradation of pro-uPA in tumor tissues may be caused by elastase released from phagocytic cells.

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Maturation of human cathepsin D in human and in transfected hamster cells

Human cathepsin D is synthesized as a larger molecular weight precursor. The synthesis and processing of this precursor has been studied in various cell lines. It has been reported that the precursor contains two N-linked oligosaccharide side chains, which can be modified to contain mannose 6-phosphate residues. These residues serve as a recognition signal for targeting of cathepsin D to lysosomes. Upon the segregation from the secretory pathway the precursor (53 kDa) undergoes proteolytic processing to mature enzyme, which consists of large (31 kDa) and small (14 kDa) subunits. A portion of the precursor escapes the targeting and becomes secreted. To examine the expression and maturation of human cathepsin D in heterologous cells we have transfected BHK cells with a vector harbouring human cathepsin D full length cDNA under the control of the SV40 early promoter. In stably transfected BHK cells we found a severalfold enhanced activity of cathepsin D. The cells synthesized precursor of human cathepsin D indistinguishable from that synthesized in human fibroblasts. In the transfected cells a portion of the precursor was secreted. Both the secreted and intracellular cathepsin D contained phosphorylated oligosaccharides. The intracellular cathepsin D precursor was processed to mature polypeptides of the same size as in human fibroblasts.

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Bernd Hovemann and Eliana Dessen

Neurological effects of the ebony mutation in Drosophila

The mutant ebony suffers from a biochemical defect that interferes with cuticle formation and in addition leads to behavioural abnormalities. The electroetinograms of ebony flies are abnormal, since they lack ON and OFF transients. The mating success of ebony is reduced which has been reported to be due to the inability to respond to either visual and/or olfactory stimuli from the female.

Ebony mutants are unable to incorporate β-alanine into the cuticle. The detection of elevated Dopamine- and β-alanine but reduced N-β-alanylodopamine pools in young flies lead to the observation that ebony mutants are defective in or devoid of N-β-alanyldopamine synthetase. We are interested in the molecular link between the observed alteration of the Dopamine pool of ebony flies and their behavioural abnormalities particularly the reported visual and olfactory blindness.

In order to be able to understand the biochemical consequences of the ebony mutation it is necessary to know where in the animal and at what time in development the gene product is present. We cloned the ebony gene and determined the gene structure. A major transcript of 3.2 kb is present throughout development. It's single open reading frame encodes the putative ebony protein of 100 kDa molecular weight. For the identification of the sequence elements that regulate ebony expression, we first determined the extent of 5' upstream sequence that is sufficient for a complete rescue of the wild type phenotype by P-factormediated germ line transformation. At present we use this ebony upstream regulatory sequence to drive ebony specific β glactosidase expression. This will be used to develop x gal staining in those cells that express the gene and thus show us if it is eventually active in certain parts of the brain.

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Isolation, Purification, Characterization and Kinetic Studies of Enolase from *Streptococcus mutans* FA-1 (*S. rattus* FA-1)

One aspect in a broad spectrum of possible cariostatic reaction mechanisms of Fluoride, are its interactions with the metabolism of oral bacteria[1,2]. Information is lacking on the mechanisms and kinetics of Fluoride inhibition of essential enzymes of the glycolytic pathway of the relevant bacteria. In this work, a new isolation and purification method of Enolase from *S. mutans* and its characterization is presented. The enzyme has been isolated in a monomeric (22 kDa) and in a dimeric form (49 kDa). Fluoride inhibition kinetics have competitive character, while Phosphate in concentrations above 2 mM alters in the presence of 0.5 mM Fluoride the inhibition kinetics from competitive to non-competitive inhibition. 2 mM of Phosphate without Fluoride have a slight stimulatory effect on the enzyme. Monofluorophosphate has a strong non-competitive inhibitory effect on the enzyme.

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Schwefel und Phosphor als Sonden der makromolekularen Strukturanalyse

Die anomale Röntgenstreuung des Schwefels und des Phosphors ist in der Nähe der K-absorptionskanten bei Wellenlängen um 5,0 Å bzw. 5,74 Å zu beobachten. Im Bereich größter Absorptionsänderunf (d.h. in der Absorptionskante) wird der Realteil des Atomformfaktors dieser Elemente halbiert.
Die Eindringtiefe langwelliger Röntgenstrahlung in Luft beträgt nur wenige cm bei Wellenlängen um 5 bis 6 Å. Für organische Festkörper ist sie tausendmal kleiner, einige hunderstel mm. Die Beherrschung der Röntgendiffraktometrie in diesem Wellenlängenbereich ist zunächst ein technisches Problem: der Strahlweg muß weitgehend evakuiert sein, die Proben sind so dünn wie ein Blatt Papier. Ein Gerät, das auch im langwelligen Röntgenspektrum Diffraktometrie und Spektroskopie gleichermaßen erlaubt, ist am Strahl A1 des HASYLAB bei DESY aufgebaut worden (1).

Die anomale Dispersion der Röntgenstreuung des Schwefels erlaubt eine einfach durchzuführende Unterscheidung der Schwefelatome in Aminosäuren Cystein und Methionin von solchen in Sulfaten. Die Verschiebung der K-Absorptionskante beträgt 10 eV. Die Verteilung der Schwefelatome des Methionins und der Glykolipidsulfate der Purpurmembran wurden getrennt bestimmt (2).
Die Unterscheidung zwischen Cystein und den Disulfidbrücken des Cystins erscheint aufgrund der 1 eV breiten Aufspaltung der Absorptionskante möglich.
Die Dispersion der anomalen Kleinwinkelstreuung des Phosphors der großen Untereinheit des E.coli Ribosoms bestätigt die relativ mittelpunktsnahe Anordnung der ribosomalen RNS. Ebenso sind die komplementären Messungen an Schwefel durchgeführt worden, die ein Bild über die räumliche Verteilung ribosomaler Proteine vermitteln. Die Ergebnisse der Neutronenstreuung in H_2O/D_2O Mischungen werden bestätigt.

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Localization of Cytoplasmic Collagen mRNA in Human Aortic Coarctations and in Arteriosclerotic Arteries by in situ Hybridization

Enhanced synthesis and deposition of extracellular matrix components including collagen contribute significantly to arteriosclerotic changes in the arterial vessel wall. We localized cells actively synthesizing collagen by hybridizing ^{35}S-labeled RNA probes complementary to type I and III collagen mRNA with cytoplasmic mRNA in frozen sections of human aortic coarctations and in arteries with different degrees of arteriosclerosis. Aortic coarctations were chosen as a model for comparing mRNA levels in areas of high blood pressure-induced wall thickening and in unaffected poststenotic areas.

In situ hybridization revealed increased expression of type I and III collagen mRNA in intimal cells and in cells adjacent to the medial-adventitial border in the prestenotic part of coarctations. In contrast, cells of the poststenotic area showed only a very low signal. No immunohistologically detectible macrophages were seen in subendothelial areas where mRNA levels were high.

In frozen sections of human arteria renalis and iliaca, type I and III collagen mRNA was enhanced in cells in intimal plaques, in the medial-adventitial border region and in the vicinity of adventitial capillaries. Macrophages and monocytes were always found in areas of enhanced mRNA expression. Macrophages and collagen mRNA synthesis were not detected in intimal plaques of 2 of 5 arteriosclerotic and in non-arteriosclerotic arteries.

Thus, higher collagen mRNA levels occur in particular regions of high blood pressure-induced arterial wall thickening in the absence of macrophages and in arteriosclerotic plaques in their presence. The results suggest that collagen synthesis in the arterial wall is regulated at the transcriptional level.

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DCCD-induced proton shortcircuit in photosystem II from water to Q is S-state independent

Photosystem II of green plants is a transmembrane protein made up of about 15 different polypeptides. It oxidizes water at a manganese centre at the lumen side of the thylakoid membrane and reduces bound plastoquinone at the stroma side. Under excitation with a series of light flashes the manganese centre cycles through four successive redox states, called S_0-S_3. This is accompanied by proton release into the lumen which follows a pattern of 0:1:2:1 according to the transitions S_1-S_2, S_2-S_3, S_3-S_0, S_0-S_1, respectively. Reduction of bound plastoquinone involves proton uptake from the stroma, which occurs independent of the redox state at any transition. Thus photosystem II acts as a proton pump. The reaction sequence of the manganese centre with (bound) water is under intensive investigation. Liberated protons can be indicative of oxidation of bound water, but also of an electrostatic response of aminoacid side chains to the abstraction of an electron from the catalytic centre.

We asked for the routing of protons between the catalytic centre and the thylakoid lumen. We found that covalent modification by DCCD of two polypeptides (20 and 24 kDa) shortcircuited the proton pumping activity of photosystem II (1). Protons were under these conditions no longer released into the lumen, but instead they crossed the membrane within the protein to protonate the reduced quinone. Interestingly, the shortcuit was independent of the respective redox transitions, i.e. at any transition a constant fraction (about 50 %) of protons were redirected across the membrane (2). This suggested that any proton released by water oxidation originated from the very neighbourhood of the manganese centre and not from peripheral aminoacid side chains.

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Klonierung und Sequenzierung der Regulatorgene (scrR) für den Sucroseabbau von Klebsiella pneumoniae und pUR400

Die Gene für den Sucrosemetabolismus können bei Enterobakterien chromosomal oder plasmidkodiert vorliegen. Entsprechend zeigen verschiedene Arten einen konstanten (Klebsiella) oder einen variablen Sucrosephänotyp (E.coli, Salmonella). In vorausgegangenen Arbeiten (1-4.) konnte gezeigt werden, daß es hohe Übereinstimmungen zwischen dem chromosomal kodierten Sucrosestoffwechel von K.pneumoniae und dem auf dem konjugativen R`-Plasmid pUR400 lokalisierten gibt. Bei beiden ist die Aufnahme des Substrates PTS- abhängig. Beide zeigen die gleichen induzierbaren Enzymaktivitäten für ein membrangebundenes Transportprotein EII^{scr} (Gen scrA), eine lösliche Invertase (scrB) und eine Fruktokinase (scrK). Die Enzyme zeigen identische Spezifitäten. Beide Systeme sind induzierbar durch Fruktose, Sorbose, Sucrose oder Fruktose- haltige Oligosaccharide wie Raffinose. Die für einen Repressor kodierende Regulatorgene (scrR) von beiden Systemen wurden subkloniert und sequenziert. Das scrR-Gen von K. pneumoniae liegt auf einem 2,3 kb BamHI/ClaI- Fragment. Das scrR- Gen von pUR400 liegt auf einem 1,9 kb BamHI/SphI-Fragment. Die Größe der offenen Leseraster beträgt jeweils 1002bp entsprechend 334 Aminosäuren. Beide Gene sind hoch homolog: Auf DNA-Ebene beträgt die Homologie 85,5%, auf Aminosäureebene 91,3%. Die Repressor/Operatorpaare zeigen hohe Kreuzspezifität und können einander komplementieren. Beide Proteine zeigen im N-terminalen Bereich Homologien zu anderen DNA-

bindenden Proteinen (z. B. galR, deoR). Die Sequenzierung so nahe verwandter Gene bietet die Möglichkeit, Leserasterfehler bei der Feststellung der Sequenz sofort zu erkennen (5.).

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Type VIII Collagen: Structural Studies on a Pepsin Fragment and Occurrence in Different Tissues

Type VIII collagen was purified from peptic extracts of bovine Descemet's membranes. Rotary shadowed fragments were visualized as rods with an average length of 132 ± 22 nm, thus being approximately half as long as monomers of the interstitial collagens. Segment long spacing crystallites could be precipitated with ATP. The triple helical structure was remarkably thermally stabile, the denaturation temperature being 4°C higher than that of type I collagen. As reported previously [1], two chains (A and B) were liberated in a ratio of 1:2 upon denaturation without reduction. The isolated chains proved remarkably resistant to trypsin and other proteases. Analysis of amino acid sequences of peptides of the A chain revealed that the normal triplet structure typical for collagens was interrupted several times by the sequence Gly-X-Gly.

Type VIII collagen was detected in peptic extracts of bovine fetal calf aorta and Ewing's sarcoma with the aid of polyclonal antibodies and immunoblotting following gel electrophoresis. Polypeptides of a nondegraded form with 50 kD were found in guanidinium chloride extracts from Descemet's membrane.

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Immunolocalization of cAMP-dependent Protein Kinase Subunits along the Secretory Pathway. Specific Accumulation of the Catalytic Subunit in Secretory Granules of Parotid Acinar Cells after Stimulation of Rats with Isoproterenol

Ultrastructural localization by an immunogold procedure detected the regulatory subunit RI and the catalytic subunit C of cAMP-dependent protein kinases in zymogen granules of the rat pancreas. In contrast, the secretory granules of parotid acinar cells showed a strong immunolabelling for the regulatory subunit RII but only negligible labelling for the RI- and C-subunit. C- and RI-immunoreactivity was also detected in the cisternae of the RER of the pancreas cells, RII-immunoreactivity in the RER-cisternae of parotid acinar cells. Both, RII- and C-immunoreactivity, were further localized in the Golgi complex of parotid cells.

Injection of rats with isoproterenol led to an increase of C-immunoreactivity in the parotid secretory granules which became maximal at 24 h and was again decreased at 37 h after stimulation. The concentration of gold particles indicating the presence of the C-subunit in the granules was amplified by repeated injection of isoproterenol. These results were confirmed by ELISA-determinations of the protein kinase subunits in isolated secretory granules. Our results suggest the existence of a cytoplasmic and a secretory form of cAMP-dependent protein kinase subunits in which the production of the exocytotic form is under regulatory control.

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Allosteric regulation of the $GABA_A$-receptor complex by barbiturates and steroids

Barbiturates are known to modulate central nervous functions via the $GABA_A$ receptor. Recently, experimental data have accumulated indicating that steroids may act similarly. Therefore, we examined the effects of pentobarbital and 3α-hydroxy-5α-pregnane-20-on (HPO) on the binding of ^{3}H-muscimol in vitro. Sprague-Dawley rats were killed by decapitation. The brains were quickly removed and frozen at -80 °C. After thawing, the tissue was homogenized in a triethanolamine / sucrose buffer (pH 9.0) and centrifuged at 1,000 g. After a centrifugation at 20,000 g, the pellet was resuspended in 50 mM POPSO (Pipearzine-N,N'-bis[2-hydroxypropane sulfonic acid], pH 7.4) and recentrifuged. The tissue was freeze-thawed again and centrifuged 3 times (30,000 g). The resulting membrane fraction was stored frozen at -80 °C until assayed for the specific binding of ^{3}H-muscimol in the absence or

presence of pentobarbital (100 µM) or HPO (1 µM).
Using this method, the in-vitro binding of ^{3}H-muscimol was increased to 185 % in the presence of pentobarbital and to 142 % by HPO. The effect of pentobarbital was restricted to the frontal brain. Shaking of the crude homogenate, preincubation at 37 °C or the use of other buffers instead of those mentioned above abolished the increase in muscimol binding.

The data show that the stimulatory effect of pentobarbital or HPO on the binding of ^{3}H-muscimol is restricted to the frontal brain and highly dependent on the preparation of the brain tissue and the buffers used for incubation.

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Anion Binding Site in Red Blood Cell Membranes

A great deal of structural and functional information about anion transport in the red blood cell membrane has been obtained through chemical modification methods. A class of anion transport inhibitors which has been extensively used is the class of stilbene disulfonate. Studies with these compounds have led to the implication of the 96 000 kDa polypeptide (band 3) in the mechanism of anion exchange through the red blood cell membrane[1-2]. Another class of anion transport inhibitors which is first used in this laboratory is the class of arginine specific reagents[3,4]. The site of action of these compounds has been found not to be identical to the site of action of both the covalently and non-covalently binding site of the stilbene-disulfonate derivative H_2DIDS. Our recent results have also shown that the reversibly binding arginine specific reagent 4-hydroxy-3-nitrophenylglyoxal (HNPG) is a competitive inhibitor of anion transport in the red cell membrane[5]. In order to get some information about the local environment and some chemical properties of these essential arginines we have synthesized a series of phenylglyoxal derivatives with different side chains and their inhibitory character on anion transport has been investigated. Our results have shown that the inhibitory character of the phenylglyoxal derivative with hydrophilic side chains, such as $-SO_3^-$, -OH, and -COOH groups differ from the derivative containing hydrophobic side chains. Our results may suggest the existance of at least two different arginine residues that participate in the anion binding site.

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Binding of Proteins to Specific Target Sites in Membranes Measured by Total Internal Reflection Fluorescence Microscopy

A new quantitative technique for determining the binding of proteins to membranes is described. The method is based on a combination of total internal reflection fluorescence microscopy (1) and the preparation of supported planar bilayers (2). Specific and reversible binding of a fluorescence labeled monoclonal antibody to lipid haptens which were embedded in supported bilayers (3) has been measured by this technique and compared to binding experiments which were conducted on membrane vesicles in solution (4). A consistent set of equilibrium binding constants and kinetic parameters have been determined and used to provide a more detailed molecular picture of the antibody-lipid hapten reaction. Estimates demonstrate that this technique is capable to measure a broad range of binding costants (down to about 10^4 M^{-1}) by using only small amounts of ligand and receptor.

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Substitution of the Cysteinyl Residue in Subunit b of the ATP Synthase of Escherichia coli

The ATP synthase (F_1F_o) of E. coli (EC 3.6.1.34) consists of the two oligomeric components F_1 and F_o. The water-soluble part F_1 carrying the catalytic centres is bound to the membrane-integrated F_o portion, which functions as a proton channel. F_o is composed of subunits a, b, and c with a proposed stoichiometry of 1:2:10±1, respectively.

Subunit b (156 amino acids) is anchored in the membrane with its hydrophobic N terminus (about 30 amino acids) leaving the major part of the protein exposed to the cytoplasm. Several lines of evidence support the view that the hydrophilic C-terminal part of subunit b is involved in the interaction with F_1 (1). The only cysteinyl residues of the F_o complex are located in the N-terminal regions of the two copies of subunit b at position 21, respectively. Chemical modification of Cys-21 results in reduced H^+-translocating activity leaving the F_1-binding capacity untouched (2).

To further investigate the role of this residue for the function of F_o amino acid substitutions for Cys-21 (Ser, Thr, Ala, Gly, Asp, Pro) were generated via "cassette" mutagenesis and the properties of the mutant F_1F_o complexes have been studied.

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J. Kautz and K.D. Schnackerz

Putative reaction mechanism and inhibitor studies of pig liver dihydropyrimidine amidohydrolase

Dihydropyrimidine amidohydrolase (DHPase, EC 3.5.2.2) is the second enzyme involved in the three-step degradation pathway of pyrimidine bases regulating the concentration of thymine and uracil nucleotides for DNA and RNA synthesis in proliferating cells (1). Pyrimidine analogues like 5-fluorouracil, clinically used in cancer therapy, are unfortunately rapidly degradated (2,3). DHPase seems to be the rate-limiting enzyme in human liver (4).

The enzyme was purified from pig liver according to a procedure established for the calf liver protein (5). Both enzymes consist of 4 subunits with 4 moles of tightly bound zinc per mole of protein. Log V_{max}/K_m versus pH data result in a pK of 7.6. We postulate that the observed pK reflects the involvement of an amino acid residue - in combination with a zinc atom - enhancing the nucleophilic attack of a water molecule on C-4 of dihydrouracil initiating the ring opening. Attempts to modify amino acid residues in the appropriate range of pK values (tyrosine, histidine, cysteine) failed. Enzymatic conversion of dihydrouracil is inhibited competitively by its reaction product N-carbamoyl-β-alanine (K_i= 1 mM, K_m (DHU)= 10.8 µM) and various structurally related compounds. Comparison of the detected K_i values indicates that the carboxyl group as well as the terminal amino function are essential for binding. The remaining β-amino group of N-carbamoyl-β-alanine can be replaced by a methylene group leading to a four-fold lower K_i value (glutaric acid monamide: K_i= 0.25 mM).

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Polymorphism of Human Prolactin in Pituitary, Serum and Amniotic Fluid

Prolactin (PRL), in contrast to peptide and glycoprotein hormones, shows size as well as charge heterogeneity. Although several groups reported different forms of PRL there are still discrepancies with respect to number and distribution pattern of the hormone forms. We investigated the whole polymophism of PRL in pituitaries, amniotic fluid and serum of lactating women and patients

with prolactinoma using gel chromatography (size heterogeneity) and isoelectric focusing (charge heterogeneity). In pituitaries (males and females) we found three forms of different molecular size as measured by RIA and by IRMA: big-big PRL of 110 000 daltons, ranging between 4-12%; big PRL (45 000 daltons, 5-15%) and monomeric little PRL (22 000 daltons, 70-85%). In sera of lactating women, however, a higher amount of big PRL (10-28%) could be detected but very little if any of the big-big form. Surprisingly, similar results were obtained in sera of patients with prolactinoma. In contrast to published data no higher molecular forms could be identified in amniotic fluid. Treatment of the higher molecular forms in pituitaries by mercaptoethanol resulted in a complete conversion of big PRL into the monomeric form suggesting a dimer product with interchain disulfide bonds. However, the big-big species remained unchainched not confirming the findings of other authors[1].

Parallel investgations of the isohormones of PRL based on different isoelectric points revealed three main and two minor components in pituitaries ranging in pI between 4.2 and 6.1. In amniotic fluid one main form (pI 4.5) was absent. These new data are of interest under the aspect of the two different sources for the hormone production: lactotroph and decidua. The possible physiological role of the isoforms of prolactin will be discussed.

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V. Kishan and W. Hillen

Molecular cloning of *trp*B Gene from *Acinetobacter calcoaceticus*

Tryptophan biosynthesis has been studied extensively in a wide variety of bacteria, which yielded information regarding the operon structure and regulation of expression. It also provided a context to compare the evolutionary relationships in bacteria. In Acinetobacter calcoaceticus, the tryptophan structural genes were mapped at three chromosomal locations, namely, trpE, trpGDC and trpFBA (1).

Our interest has been to clone and characterize the trpFBA gene cluster in Acinetobacter calcoaceticus. By homologous complementation of BD413 trpB18 mutant, three recombinant plasmids were obtained. None of these could complement BD413 trpA23 mutant, although the size of the inserts varied in between 9.5 kbs and 19 kbs. By deletion mapping trpB gene was located in a 3.3 kb region. Nucleotide sequence analysis revealed the trpB gene sequence. The encoded protein is homologous to the respective structures from other organisms. Models of regulation of expression of the trp genes in this organism are discussed.

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Specific Binding of Phlorizin to Mammalian Catalase

In an attempt to purify the Na^+/D-glucose cotransporter from pig kidney brush border membrane fraction using affinity chromatography with phlorizin columns (3-amino-phlorizin coupled to sepharose), a major protein of 58 kDa was bound to the column in the presence of 100 mM Na^+ and was specifically eluted with 1 mM phlorizin. *Comparison of the amino acid sequence of three tryptic peptides of this protein and its enzymatic activity identified this protein as catalase (EC 1.11.1.6). The presence of phlorizin had no effect on the catalase activity. Because catalase has a NADPH binding site of yet unknown function, we examined the effect of NADPH on binding of catalase to phlorizin. We found that catalase bound to phlorizin column can be eluted with as low as 50 µM NADPH. In additional experiments we used 4 µM ^{3}H-azidophlorizin (18 Ci/mmole) to label the enzyme catalase. NADPH at a concentration of 1 µM protected catalase from photo-labeling to about 50 %. After tryptic digestion of labeled catalase and reversed phase HPLC we detected one major radioactive peptide which was absent in the NADPH-protected sample. We presently try to isolate and to sequence this peptide and want to see whether it is part of the NADPH binding site in catalase.*

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The ß-domain of Neisseria IgA Protease: Application as a Protein Export Vehicle for Gram negative Bacteria

Cloned gonoccocal iga genes have the unique ability to direct extracellular secretion of IgA protease from gram negative bacteria like E.coli and Salmonella species (1). Mature IgA protease is initially synthesized as a large precursor which harbours all nessessary export functions (2). A typical amino terminal signal peptide is assumed to initiate inner membrane transport while the large carboxy terminal ß-domain mediates transport through the outer membrane (3).

In order to investigate the unique transport properties of the ß-domain ($Iga_{ß}$) we constructed hybrid proteins with cholera toxin B subunit (CtxB) and analysed their targetting behaviour in Salmonella. CtxB-$Iga_{ß}$ hybrids were found to assemble with the outer membrane. They were not detectable in other cellular compartments. Immunolabeling of whole cells with anti-CtxB serum revealed successful translocation of the CtxB-moiety to the surface of the outer membrane. Translocated CtxB was accessible to trypsin in intact cells and could be cleaved off from its 'membrane anchor' by incubation with purified IgA protease when a specific cleavage site was present between the fusion partners. Surface exposition of CtxB was quantitatively monitored by whole-cell-ELISA. The translocation rate increased several fold when the two cystein residues of CtxB were substituted by glycins or when bacteria were grown under reducing conditions. We conclude that formation of intramolecular cystein bonds may lead to a globular protein structure which blocks further translocation.

The IgA protease ß-domain may provide a shuttle system suitable for export of proteins by gram negative bacteria. Applications may include serum diagnostics, the construction of new live oral vaccines and the extracellular production of proteins of biotechnological interest.

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A vacuolar-type H^+-ATPase and an H^+/K^+-antiport as electrogenic K^+-pump in an insect plasma membrane.

An ATPase has been purified from the K^+-transporting globlet cell apical membrane of tobacco hornworm midgut; the enzyme is inhibited by NEM, but not by azide or vanadate, and the apparent relative molecular masses of its subunits are 67, 56, 43, 28, 16 K (1). Antibodies directed against both vacuolar-type ATPases of plant tonoplasts and of bovine chromaffin granules showed cross-reactivity with corresponding subunits of the purified goblet cell ATPase. Hence the enzyme is a vacuolar-type ATPase.

Substrate and inhibitor specificities of this enzyme are similar to those of the ATP-dependent electrogenic H^+-transport found in vesicles from this membrane (2). These same vesicles also have an ATP-independent, amiloride-sensitive H^+/K^+-antiport. Our results imply that the goblet cell H^+-ATPase sets up a proton motive force which drives the H^+/K^+ antiport producing net electrogenic K^+-flux from the cytosol to the midgut lumen.

This system provides the first example of a vacuolar-type ATPase as a permanent constituent of a plasma membrane. It is also the first example of an H^+-ATPase driving secondary active transport across an animal plasma membrane.

(supported by the DFG, HW 698/2-1)

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W. Kleinow and K. Wißling

Keratinlike Proteins from the Integument of the Rotifer Brachionus plicatilis

The lorica and other parts of the rotifer integument contain an intrasyncytial lamina of proteinaceous material. This material proves to be resistant against protein dissolving agents such as sodium dodecyl sulfate (SDS) or 8 M urea even if heated to 100 °C. This property has been used to purify lorica material from the rotifer Brachionus plicatilis by dissolving all other rotifer tissues by such agents and precipitating the remaining loricas. The purified loricas were afterwards dissolved by treating them simultaneously by SDS and dithiothreitol (DTT) or by 8 M urea and mercaptoethanol (1, 2).
Whereas intact lorica material is only slightly hydrolysed by proteinases, the lorica proteins are fastly degraded by proteinase K (EC 3.4.21.14) and pronase E (EC 3.4.24.4) after dissolving the lorica structure by the above mentioned treatments. In SDS polyacrylamide gel electrophoresis the lorica proteins separate into two main bands: a double band corresponding to protein of m.w. 53 +/- 1 kDa and a single band of 43 +/- 1 kDa, and some minor bands. After blotting on nitrocellulose and incubating with polyclonal antibodies against vertebrate keratine two out of these bands were found to be highly specifically labeled: one out of the minor bands (corresponding to 64 kDa) and the upper part of the slower moving main band corresponding to a protein of about 54 kDa.
The chemical properties of the lorica proteins together with the immunological evidence strongly indicate that the integument of rotifers is composed from keratinlike intermediate filaments. This structure may therefore provide another example of occurrence of intermediate filament material in invertebrate animals (3, 4).

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L.Klewes and P.Prehm

The eukaryotic hyaluronate synthase

The eukaryotic hyaluronate synthase is located at the inner side of the plasma membrane and it synthesizes hyaluronate directly into the extracellular matrix (1), where it is bound to rezeptors (2). The prokaryotic synthase has been isolated and characterized (3).

The eukaryotic synthase was isolated by affinity chromatography on insolubilized antibodies, directed against the streptococcal synthase. The enzyme activity could be enriched 1400fold from digitonin extracts of the plasma membranes. The SDS-PAGE showed two proteins with 50 and 60 kD in equimolar amounts.

The synthase was stimulated by foetal calf serum. Labeling experiments with ^{35}S-methionine and ^{32}P-phosphate showed, that the increased activity was due to enzymeinduction and phosphorylation.

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Isolation and analysis of a genomic clone for the human Mr 46 000 mannose 6-phosphate receptor

The human Mr 46 000 mannnose 6-phosphate receptor (MPR 46) is involved in the mannose 6-phosphate dependent targeting of newly synthesized lysosomal enzymes to the lysosomes. A cDNA clone (P29) for the human MPR 46 has been isolated from a human placenta cDNA library in λgt 11 (1). The cDNA clone P29 (2463 bp) was used as a probe for screening of a human leucocyte genomic library in EMBL 3. Thirty positive clones were isolated and analysed for coding sequences by hybridization with oligonucleotides that were deduced from the cDNA. Two overlapping clones were found to contain the entire coding sequence. Clone C12-P1 (18 kb) contains the 5' untranslated region of the cDNA and about 80% of the translated sequence (bp -145 to bp 711). Clone A10-4 (12 kb) contains about half of the translated region and the 3' untranslated region (bp 344 to bp 2283 of the cDNA). The entire size of the genomic MPR-46 DNA is about 19 kb. It is divided into seven exons. The intron-exon structure will be presented.

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The heat-shock response in a photoautotrophic cell culture of Chenopodium rubrum

Heat-shock (HS) offers an efficient system for studies on regulation of gene expression within the cell. The photoautotrophic cell culture of Chenopodium rubrum reacts rapidly to a sublethal increase of the ambient temperature to 38°C with the production of heat-shock proteins (HSP). The time of maximal expression is a characteristic property of an individual HSP as has been found by in vivo and in vitro translation indicating a differential transcription with time. An additional factor of regulation is light intensity, high light intensities (25.000 lx) reduce the temperature of induction from 38°C to 36°C or lower to achieve the same HSP accumulation. No effect of photoinhibition has been observed under these conditions. While a 32 kDa protein dominates among the in vitro translation products the dominant HSP after in vivo labeling has an apparent mol. mass of 25 kDa indicating a precursor-product relationship. Sequence comparison of this particular protein

shows similarity with HSP 17.5 of soybean and even more with the sequenced plastid heat-shock protein of pea[1]. Based on this evidence we assume that HSP 32 is a nuclear coded plastid protein processed into a 25 kDa product.

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Identification and Regulation of High Affinity Transporter for Choline in Synaptosomes

There is considerable evidence indicating that the accumulation of choline via a specific high affinity uptake system is the rate-limiting step for the synthesis of acetylcholine. It is one of the most interesting properties of this high affinity transport system that its capacity appears to be coupled to the neuronal activity [1]. Some recent experiments suggest that this coupling may be brought about via intrasynaptosomal messengers. Modifying the concentration of cyclic nucleotides in synaptosomes, significantly increased the rate of choline accumulation; a similar effect was induced by phorbol esters, known to activate the protein kinase C. The observed changes in the rate of high affinity choline accumulation could either result from alterations in the velocity of the transport process or via changes in the actual number of functional carriers. In experiments using tritiated hemicholinium-3 as specific probe for choline transporter we have found that the number of binding sites significantly increased upon kinase activation. In approaches to identify the carrier protein monoclonal antibodies were produced which specifically block the high affinity transport of choline. These antibodies were found to recognize a single polypeptide band (M_r= 80.000) on Western blots. By FPLC-ion-exchange and immunoaffinity-chromatography the 80kD polypeptide has been purified to homogeneity and was subsequently reconstituted in liposomes.

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Fortschritte der Spinkontrastvariation

Die Ampltituden der Streuung polarisierter Neutronen an polarisierten Protonenspins übertreffen die durch Isotopenaustausch erreichbaren Werte um das dreifache. Beim schwereren Wasserstoffisotop Deuterium ist die Polarisationsabhängigkeit der Neutronenstreuung viel geringer. Protonencluster in deuterierter Matrix sind daher im Lichte polarisierter Neutronen sehr wirksame, reversible Markierungen der makromolekularen Strukturforschung. Durch Variation der Kernspinpolarisation läßt sich an ein und demselben Präparat in weiten Grenzen jeder gewünschte Kontrast, den wir hier als Spinkontrast bezeichnen, einstellen (1).
Bei Temperaturen unter 1 K und in einem äußeren Magnetfeld von 2.5 T werden Protonenspins durch Mikrowelleneinstrahlung in Gegenwart ungepaarter Elektronen in günstigen Fällen bis zu P=0.95 polarisiert. Der Spinkontrast einer Reihe von Proteinen, tRNA und von Ribosomen wurde gemessen. Für Proteine in deuterierten Lösungsmittel verschwindet der Spinkontrast bei P=0.6. Bei tRNA ist dieses bereits bei P=0.35 der Fall.
Eine weitere Dimension der Spinkontrastvariation eröffnet sich durch die selektive Kernspindepolarisation eines dynamisch polarisierten Targets durch Übersättigung der Kernresonanz. Aus der Streuung polarisierter Neutronen läßt sich dann die räumliche Verteilung der Protonen und der Deuteronen in einem Makromolekül getrennt erfassen. Bei T < 0,2 K können Kernspinsysteme unterschiedlicher Polarisation über Tage koexistieren. Zeit genug für die Untersuchung durch polarisierte Neutronen.

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Studies on the binuclear metal center of the purple phosphatase from Red Kidney-Beans

In connection with studies on the purple acid phosphatase from beef spleen (BSPase) containing a novel type of Fe^{2+}-Fe^{3+}-cluster we also investigated the structure of the Zn^{2+}-Fe^{3+}-center of the prple phosphatase from Kidney-beans (KBPase). KBPase is a very well crystallizing dimer consisting of two identical subunits with M_r = 58200 (1,2).
The colour of the enzyme is due to a charge-transfer-band with a maximum at 560 nm which reversibly shifts to 545 nm at lower pH-values with a pK around 4. Resonance-Raman studies of KBPase revealed four respnance enhanced Raman bands at 1171, 1287, 1503 and 1606 cm^{-1} which are typical of Fe^{3+}-tyrosinate coordination.
In the presence of inhibitors like phosphate, arsenate and molybdated hypsochromic shifts take place, too, indicating a bridging of the Zn^{2+}- and Fe^{3+}-cation by the oxoanions. EXAFS-data suggest a Zn^{2+}-Fe^{3+}-distance of 30.5 pm which increases upon binding of oxoanions to 33.5 pm (3).
The removal of the Zn^{2+} by incubation with EDTA yields an inactive "semi-apoenzyme" with a visible absorption band at 521 nm. Reactivation occurs at the addition of one equivalent of Zn^{2+} or Co^{2+}, but may also be achieved by application of a 10-fold excess of Mn^{2+}. KBPase can be recon-

stituted by Fe^{2+}, too, which provides an enzyme resembling BSPAse. The exchange of Zn^{2+} shifts the absorption band to 565 nm (Co^{2+}), 550 nm (Mn^{2+}) or 544 nm (Fe^{2+}).
Native KBPase shows an ESR-signal at g=4.3 (4) originating from high-spin Fe^{3+} whereas the Co^{2+}-substituted enzyme is ESR-silent. This obviously indicates spin coupling between Co^{2+} and Fe^{3+} due to an interaction of the hydroxo-ligand situated at Fe^{3+} with Co^{2+}.
From the spectroscopic studies evidence can be obtained on the mechanism of the phosphatase reaction.

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Molecular and biochemical investigation of the ptsI gene and the gene product Enzyme I of *Staphylococcus carnosus*

A digoxigenin-labeled DNA probe, complementary to ptsH and the beginning of ptsI, was used to clone a 3.1 kb HincII/BamHI restriction fragment, which contains the ptsI gene of *Staphylococcus carnosus*, in vector pSU18.

For overexpression of the Gram-positive enzyme I in *E. coli* TG1 a 2.7 kb HincII/XbaI fragment was subcloned into pUC19, called pUC19-ptsI*Sc*.
From 20 g (wet weight) *E. coli* TG1 cells, it was possible to purified 25 mg active enzyme I to homogeneity. This result is about thirty times the amount you receive from 20 g *S. carnosus* wild type cells.
The purification procedure involved Q-Sepharose anion exchange chromatography, ammonium sulfate precipitation, acid precipitation, gelfiltration (G75), hydrophobic chromatography on Butyl-TSK and a second anion exchange step on FPLC column Mono-Q.

On SDS-PAGE enzyme I shows a single band at a molecular weight of 70 kDa. However, after cross-linking with DTBB a molecular weight of 140 kDa was determined, indicating that the protein has to be a dimer in native state.

Furthermore, it was possible to complement the *E. coli* ptsI$^-$ mutant JLT2 by the pUC19-ptsI*Sc vector* and to compare the pts sugar transport between ptsI$^-$ mutant, complemented ptsI$^-$ mutant and *E. coli* HB101.

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Are Human Heparin-Binding Lectin and Fibroblast Growth Factor Related?

The heparin-binding lectin from human placenta is isolated on the basis of its tendency to form large aggregates by gel filtration and on the basis of its affinity to heparin by affinity chromatography. The purified lectin dissociates into up to four distinct polypeptides with M_r values of 14.4, 15.0, 16.2 and 16.7 and a single isoelectric point of 9.0. Molecular heterogeneity is not due to different degrees of glycosylation, as evidenced by gel elctrophoretic analysis after extensive treatment with various endoglycosidases. Despite its similarities of affinity to heparin, molecular size and isoelectric point to the basic fibroblast growth factor (bFGF), the comparitively high yield of the lectin (approximately 1.5 mg per 100 g placenta), the occurrence of proteolytic fragmentation in the presence of heparin and the lack of homology to the amino-terminal sequence of the lectin argue against any notable relationship to bFGF. Most importantly, the lack of mitogenic activity in a commonly used bioassay with quiescent 3T3 fibroblasts rules out any EGF-like activity on cell proliferation. The heparin-binding lectin is thus clearly distinguishable from heparin-binding growth factors. By employing biotinylated heparin as labelled ligand to visualize and quantify heparin binding, hapten inhibition in a solid-phase assay reveals that except for heparin no other vertebrate glycosaminoglycan, but the sulfated fucan fucoidan can effectively reduce the Ca^{2+}-independent ligand binding. Proteolytic fragmentation by chymotrypsin in two independent assays demonstrates that a fragment of a M_r value of 7.8 still retains ability to bind heparin. The interaction of this lectin with naturally occurring heparin-like molecules may physiologically be involved in modulatory regulation of heparin-mediated processes.

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Potassium transport in Escherichia coli: Purification of the Kdp-ATPase and Reconstitution into Liposomes

The Kdp-ATPase, located in the cytoplasmic membrane of the enterobacterium E.coli is a high-affinity potassium uptake system (K_m = 2 μm). This multimeric enzyme complex consists of three subunits: KdpA (59 189 Da), KdpB (72 112 Da) and KdpC (20 267 Da) (1).

ATP hydrolysis activity is detectable in inside out vesicles and in the purified state of the enzyme; potassium translocation, however, is only measurable in living cells. To eliminate the interfering activity of other ion transport components located in the cytoplasmic membrane (f. i. proton potassium antiporter) it was advisable to reconstitute the purified enzyme complex into liposomes to detect potassium translocation across the membrane and to get an idea about the K^+/ATP stoichiometry. Unfortunately the established purification protocol (2) contains a major drawback. For the solubilization a detergent (Aminoxid) is used, which can only be removed by methods impairing the enzyme.

A new purification procedure was developed which involves solubilization by dialyzable detergents, and purification by ion exchange chromtography and affinity chromatography with dye media. Effects of different detergents on the integrity of the multimeric complex as revealed by this procedure will be presented.

A reconstitution protocol was established, where the purified enzyme was successfully reconstituted into preformed liposomes during a dialysis procedure.

The potassium specific fluorescence dye PBFI (Molecular Probes) showed a valinomycin inducible release of potassium from the liposomes. The ATP inducible release of potassium from proteoliposomes is under investigation.

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J. J. Kort and G. Koch

The Na^+, K^+,$2Cl^-$-Cotransport System in HeLa Cells and its Regulation by Atrial Natriuretic Peptide

We have investigated the characteristics of a 'loop diuretic' sensitive Na^+-K^+-Cl^--cotransport system in HeLa monolayer cells. Our results are consistent with a Cl^--dependent Na^+, K^+ cotransport via the cell membrane in Hela cells(1) . $^{86}Rb^+$- and $^{22}Na^+$ influx studies led to a calculated ratio of 1 Na^+: 1K^+ transported together. Bumetanide (K_i= 0.8 µM) and piretanide (K_i= 3 µM) inhibited the cotransporter in a dose dependent manner. In addition, (^{3}H)-bumetanide binding to intact HeLa cells revealed a number of 103000 binding sites/cell. 30% of total $^{86}Rb^+$ influx into HeLa cells account for the bumetanide-sensitive fraction under isotonic conditions. Hyperosmolarity, established by mannitol addition markedly stimulated both, total and bumetanide-sensitive $^{86}Rb^+$ uptake into HeLa cells(2). However, Atrial Natriuretic Peptide (ANP) could reduce the stimulatory effect of hypertonicity on the bumetanide-sensitive fraction of $^{86}Rb^+$ influx into HeLa cells. Binding studies with (^{125}I)-ANP to HeLa cells revealed a number of 29000 binding sites/cell. Furthermore, ANP binding to HeLa cells led to an elevation of cellular cyclic GMP. In contrast, studies with the ß-adrenergic agonist isoproterenol and 8-Br-cAMP showed no effect on the bumetanide-sensitive $^{86}Rb^+$ influx. Our results indicate (1) a Na^+,K^+,$2Cl^-$-cotransport system in HeLa cells is inhibited by ANP-mediated elevation of cellular cyclic GMP and (2) hormonal control of the cotransporter in Hela cells is not dependent on ß-receptor agonists and elevation of cellular cyclic AMP.

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Rumiana Koynova and Hans-Jürgen Hinz

Metastable Behaviour of Saturated Phosphatidylethanolamines: A Densitometric Study

The thermotropic phase behaviour of saturated L_α-PE's with 12-18 carbon atoms per chain was investigated using differential densitometry. Metastable behaviour was observed for all compounds; they exhibit a phase sequence of $L_c \rightarrow L_\alpha$ in the first heating and $L_\beta \rightarrow L_\alpha$ in all subsequent heating cycles. No depedence on acyl-chain length was found for the volume changes in the $L_c \rightarrow L_\alpha$ transition, while increasing volume changes with increasing acyl-chain lengths were established for the second transition. The phenomenon of constant volume changes for the transformation of the highly ordered crystalline to the fluid phase for all PE's should be ascribed to a combined chain melting and hydration process. The calculated values for the volume changes of the hypothetical $L_c \rightarrow L_\beta$ transition converge to zero at a chain length of ~ 22, i.e. no metastability should be detected beyond this chain length. These results are in good agreement with those found in DSC-, X-ray- and IR-studies.

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Dicyclohexylcarbodiimide blocks partial activities of Na^+/K^+-ATPase associated with the E_2-but not with the E_1-conformation

The mechanism of the cation transport by the sodium pump of animal cell membranes is generally described by an oscillation between the E_1 and E_2 conformational states (Albers-Post model). The E_1-conformational state of Na^+/K^+-ATPase (EC 3.6.1.37) catalyzing the export of Na^+ out of the cell is characterized by a high affinity ATP binding site, occlusion of Na^+ into the phosphorylated enzyme and a Na^+-ATPase activity. The E_2-conformation catalyzing the import of K^+ into the cell occludes K^+, has a K^+-activated phosphatase activity, carries out the ouabain-supported backdoor phosphorylation of the enzyme and contains a low affinity ATP binding site, whose occupation leads to the de-occlusion of K^+. Carboxylic acid residues in the C-terminal transmembranal part are candidates to participate in the cation transport.

Dicyclohexylcarbodiimide (DDCD), which interacts with carboxyl groups in an hydrophobic environment, has been shown to inactivate Na^+/K^+-ATPase [1] and to label the 56 000 Da tryptic fragment of the catalytic α-subunit [2]. Since the inactivation by DCCD is partially protected by Na^+ and K^+, we studied in more detail the specificity of interaction of DCCD with the enzyme.

Inactivation of Na^+/K^+-ATPase let the partial activities of the E_1-conformation (phosphorylation of the high affinity ATP binding site with CrATP; Na-ATPase activity) unaffected. On the contrary, E_2-associated partial activities (occlusion of $^{86}Rb^+$; K^+-activated phosphatase;

ouabain-supported phosphorylation from inorganic phosphate) decreased in parallel with the overall reaction. However, binding of $Co(NH_3)_4ATP$ to the low affinity ATP binding site [3] remained unaffected. We therefore conclude that DCCD lets the ATP binding sites unaffected and seems to interact preferably with the E_2 conformational state.

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Recombinant Human Kunitz-type Elastase-Inhibitor Variants

Clinical studies have shown that the serious consequences of the release of large amounts of lysosomal elastase in septicemia and other severe inflammatory processes may be restricted by the therapeutic application of elastase inhibitors[1]. But several reasons argue against the application of the only natural human elastase inhibitor, α_1-proteinase inhibitor (α_1-antitrypsin). A promising approach to therapeutically valuable elastase inhibitors is the modification of reactive sites of small human proteinase inhibitors.

We constructed several genes coding for single- and double-headed elastase inhibitor proteins that are variants of human bikunin[2], the Kunitz-type inhibitor subunit of inter-α-trypsin inhibitor. Using fusion protein constructs, a rapid evaluation of inhibitor specificity and activity could be achieved with each design.

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H. Krell and E. Dietze

Reversal of Hemodynamic Responses to Leukotriene C4 by Isoproterenol and Dibutyryl Cyclic AMP

Responses of isolated perfused rat liver to leukotriene C4 (LTC4) include rise of portal pressure (1) or decrease in perfusate flow (2), decrease in oxygen consumption, and increase in hepatic glucose and lactate efflux and lactate/ pyruvate ratio in the perfusate, and a small decrease in bile flow. The metabolic responses have been ascribed to a direct effect on hepatocytes (1,2). We have studied the effects of isoproterenol (ISO, 1μM) and dibutyryl cyclic AMP (20μM) (DB-c-AMP) in order to scrutinize this hypothesis. ISO alone had no effect on oxygen consumption and portal pressure, and only small changes in metabolite release were observed (3). However, ISO reversed the effects of LTC4 on respiration and portal pressure while bile flow and metabolite efflux were reversibly stimulated. The same changes were observed upon withdrawal of LTC4. DB-c-AMP mimicked the hemodynamic effects of ISO while a biphasic response of bile flow was observed: a reversible decrease was followed by a sustained increase. The increase in respiration was correlated with the decrease in portal pressure upon addition of ISO or DB-c-AMP and upon withdrawal of LTC4. The corresponding release of glucose was correlated with the increase in oxygen consumption upon both ISO addition and withdrawal of LTC4. These results are discussed in terms of LTC4-induced microcirculatory redistribution of perfusate flow. Since in the presence of nitroprusside (50μM) the effects of LTC4 and their reversal were diminished, a vascular site of action can be assumed. Accordingly, the accompanying metabolic responses are consistent with gradual changes in oxygen supply to parts of the liver.

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An Improved Procedure for the Purification of Hepatic Lipase from Rat Liver Homogenates

Hepatic lipase (HL) is present in liver, adrenal glands and ovaries. Its activity is characterized by an alkaline pH optimum, salt resistence and binding to heparin. The enzyme is synthesized and secreted by liver parenchymal cells and associated with the plasmamembranes of endothelial cells. HL displays triacylglycerol, diacylglycerol, monoacylglycerol lipase and phospholipase A_1 activities, and is presumed to function in the clearance of triglycerides, phospholipids and cholesterol from plasma lipoproteins. The enzyme has been purified to homogeneity from rat liver homogenates [1]. Cloning revealed a molecular weight of 53 222 for the unglycosylated protein and two potential sites for N-glycosylation [2].

We have developed an improved procedure for HL purification from rat liver homogenates. The modified method results in an about 1.6-fold higher specific catalytic activity and approximately 4-fold higher yield when compared to [1]. The purification steps include: 1.) homogenisation in a buffered medium containing heparin and o.5 M NaCl; 2.) preparation of a crude extract by centrifugation; 3.) chromatographies in series on octyl-Sepharose CL-4B, heparin-Sepharose CL-6B and concanavalin A-Sepharose; 4.) concentration on heparin-Sepharose CL-6B; 6.) gel filtration on Ultrogel AcA-34. For protein quantitation, the purified material had to be concentrated on heparin-Sepharose CL-6B.

With the new purification device, the enzyme was purified approximately 79 000-fold over the homogenate, yielding about 41% of the starting activity, i.e. 22 U (defined as µmol free fatty acid/h per mg protein) of purified enzyme per 1 g rat liver. In a reaction system containing 2.5 mM sonicated triolein, 0.004% (w/v) Triton N-101 and various cofactors required for optimal catalytic activity [3], purified HL displayed 23 000 ± 950 U/mg protein. Upon chromatofocusing, HL activity was eluted at pH 6.1 - 6.3. SDS polyacrylamide slab gel electrophoresis of purified rat HL revealed a molecular weight of 63 300 ± 2 900 (n=5). This is in line with the molecular weight of 60 000 - 62 000 described for HL from rat liver perfusates [4].

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Structure of the human arylsulfatase a gene

Arylsulfatase A (ASA) is a lysosomal enzyme the deficiency of which causes metachromatic leukodystrophy in humans. An ASA cDNA-clone was isolated from a human testis cDNA library. The ASA cDNA encodes a polypeptide of 507 amino acids, which has arylsulfatase A activity and is transported in a mannose 6-phosphate dependent manner to lysosomes when expressed in BHK-21 or COS cells (1).
ASA cDNA hybridizes to three mRNA species of 2.0, 3.7 and 4.8 kb which arise from the use of different polyadenylation signals. A genomic clone of 14 kb of human ASA was isolated from a human genomic library in EMBL-3. Analysis of the clone revealed a size for the ASA gene of about 3.2 kb. It contains 8 exons (102 - 316 bp in size), separated by 7 introns (74 - 311 bp). S1 nuclease mapping shows that transcription initiates about 360 bp upstream of the start codon. A fragment encompassing 400 bp of the flanking sequence upstream of the transcription initiation site shows promoter activity when it was transiently expressed in COS cells using the bacterial chloramphenicol-transferase as a reporter gene. This putative promoter region shows 4 potential Sp1-binding sites but lacks a typical TATA box sequence.

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Comparison of the substrate-specifities and homologies between 6-phospho-ß-glycohydrolases from Gram positive and Gram negative bacteria

Many Gram positive bacteria such as Staphylococcus aureus take up the disaccharide lactose via the phosphotransferase system (PTS). During the translocation through the membrane the sugar is phosphorylated at the C6-atom of the galactoside part by the specific enzyme II. In the cytoplasma an enzyme is necessary to hydrolyse the disaccharide into two monosaccharides which then are available for energy metabolism.
In the case of lactose-6-P accumulated by S. aureus the splitting enzyme is the 6-P-ß-galactosidase (1).
The Gram negative E. coli possesses a kryptic operon that codes for the enzymes needed to transport and hydrolyse aryl-ß-glucosides (2). The 6-P-ß-glucosidase B is the enzyme that splitts aryl-ß-glucosidephosphates into glucose-6-P, which then enters the glycolysis, and the aryl-residue.

Comparisons of the aminoacid-sequences of the 6-P-ß-galactosidases of S. aureus, S. lactis and L. casei show great similarities between the three proteins. The homology between the enzymes of S. aureus and S. lactis is higher than to L. casei. All Gram positive 6-P-ß-galactosidases show homology to the 6-P-ß-glucosidase B of E. coli, particular between three highly conserved sequence-parts.
Similaritiers are also found on the level of substrate-specifity between the 6-P-ß-galactosidase of S. aureus and the 6-P-ß-glucosidase B of E. coli. Both enzymes are able to split the same substrates over a wide range. The K_M-values for different substituted phosphoglucosides and phosphogalactosides are similar but the v_{max}-values differ strictly.
The 6-P-ß-glucosidase B can possibly distinguish between the ortho- and para-position of the benzenemin of the aglycon. The ortho-derivates are hydrolysed much faster. One would assume that the substrate-binding sites of the two 6-P-ß-glycohydrolases may be conserved but the proteins possible differ in case of the catalytic sites.

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Early light-inducible proteins (ELIPs) of barley: Two gene families sharing high homology

ELIPs are characterized by a light-induced transient appearance of their proteins and respective mRNAs in etiolated and green plants. Coded for in the nucleus and localized in the thylakoid membranes they are ideal model systems for the study of intracellular protein transport. In barley two gene families have been found coding for at least 4 precursors of 24 to 27 kDa and at least 4 of 16.5 to 18 kDa. Seven clones have been sequenced and are compared. All have amino terminal transit sequences, however, these are not identical to each other and show comparatively low degrees of homology between the families. Although different in size two of the low molecular mass precursors are processed into products of identical length. The coding regions of both families have a high degree of homology. Large and small ELIPs are distinguished by an insert of about 30 amino acids between the transit peptide and the region shared by all ELIPs. In agreement with the transient appearance are PEST sequences in the large ELIPs. The homologous region is characterized by three transmembrane regions two of which, the first and third, are almost identical sharing also homology with the LHC I and LHC II gene families. This characteristics might help to establish a function analysis.

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The endogenous regulation of the maltose regulon is affected by *malK* and *malI*

The maltose regulon of *Escherichia coli* comprises several operons that are under common control of the MalT activator protein. Five *mal* genes code for a binding protein-dependent transport system specific for maltose and maltodextrins. MalK, one of the subunits of this transport system is not only essential for transport but also plays a role in regulation. We isolated two classes of *malK* mutants: the first class exhibits unaltered regulation but is inactive in transport; the second class is still active in transport but constitutivly expressed. The mutations were localized by sequencing.

For a *malK-lacZ* constitutivity to be exerted the function of an additional gene product, MalI, is necessary. MalI exhibits high homology to the repressor proteins GalR, CytR and LacI.The amino-terminal half of the protein exhibits significant homology with MalK. The transcriptional start of *malI* was determined.In the promoter region of *malI* we found two perfect direct repeats of 14 base pairs with two fold symmetry indicating their possible role as operator sites.The expression of *malI* itself is autoregulated. Furthermore, the expression of the *malK-lacZ* fusion is strong decreased in the present of the cloned operator sites. Upstream to *malI* we observed a divergent open reading frame that extended to the end of the sequenced DNA. The transcription of this yet unknown gene should be regulated by MalI, since MalI recognize the downstream located two operator sites.

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Disturbance of the Membrane Microenvironment by Detergent Changes Intermediate Transfer Efficiency and Reaction Coupling in Cytochrome P450XVII-Catalysed Androgen Formation

The gonadal cytochrome P450XVII functions as the substrate- and oxygen acceptor and activation site in a system (consisting of the P450 and a NADPH-P450 reductase), that catalyses the hydroxylation of progesterone in the 17α-position and the cleavage of the resulting 17α-hydroxyprogesterone, yielding androstenedione as the product. It can therefore be regarded as a bifunctional steroid hormone synthesizing enzyme[1,2]. The intermediate of this reaction sequence, 17α-hydroxyprogesterone, can be specifically retained by the membranes of the smooth endoplasmic reticulum; this pattern facilitates the efficient intermediate transfer within the androgen biosynthetic process by compartmental restriction and limitation of intermediate diffusion[1,2].

Reaction coupling in such a bifunctional system is sensitive towards changes of membrane structure. If testicular microsomal membranes are solubilized with 0.4, 0.8 or 1.6% v/v Triton CF54, respectively, and the extracts incubated (final CF54 concentrations 0.04, 0.08 or 0.16%, respectively) with different concentrations (0.5 to 4.0 μM) of the substrate, progesterone, and with 130 μM NADPH as the cosubstrate, the ratio of intermediate / product formation rates increases from 1.7 ± 0.5 (mean ± SD, n=12) to 2.4 ± 0.7 and 3.9 ± 1.4 with 0.04, 0.08 and 0.16% CF54, respectively, under initial rate conditions (1.6 with unsolubilized microsomes). In parallel, the molecular activity of the enzyme system decreases from 1.56 ± 0.44 min^{-1} (nM catalytic cycles × min^{-1} × nM $P450^{-1}$) to 1.24 ± 0.20 min^{-1} and 0.48 ± 0.08 min^{-1} with 0.04, 0.08 and 0.16% CF54, respectively (3.8 min^{-1} with unsolubilized microsomes). These results clearly indicate that modification of the microenvironment of this membrane-bound P450 system may affect not only the total activity[3] but likewise the specific retention and efficient transfer of the intermediate probably by disturbance of enzyme-enzyme interactions and partial uncoupling of the coordinated sequence of oxygen attack reactions on the steroid molecule[4].

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Single Site ATP Hydrolysis Catalyzed by the ATP-Synthase from Chloroplasts

ATP-synthases from the F_oF_1 type contain several nucleotide binding sites. It has been proposed that there are at least three binding sites which have catalytic properties and which work cooperatively. The ATP-synthase from chloroplasts was brought into the active, reduced state, and by a washing procedure the nucleotide content of the enzyme was reduced. The rate of ATP hydrolysis was investigated in the concentration range between 2.5 nM and 1 mM ATP and an enzyme concentration of 20 nM. ATP hydrolysis is observed also at the lowest substrate concentrations; i.e., under conditions where only one catalytic site is occupied ("single site conditions"). At concentrations below 100 nM, the rate depends nonlinearly on the ATP concentration. Possibly, this indicates an interaction between different nucleotide binding sites.

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R. Lange and R. Hengge-Aronis

Characterization of Carbon-starvation induced lacZ Fusions in E.coli

The carbon-starvation stimulon of E.coli is largely uncharacterized on the molecular level.
We isolated random lacZ fusions exhibiting increased β-galactosidase activity on plates containing 0.02% glucose compared to 0.4% glucose (XGal as an indicator). Since fusions to catabolite sensitive operonss such as mal or mgl, react positively in this assay, a Δcya strain was used for isolation of fusions induced by carbon starvation.
Four such fusion strains have been isolated so far. In one strain (ϕ10), a ten-fold induction of β-galactosidase occurred, at least 3 hrs after the onset of starvation. ϕ10 maps at 63.5' on the E.coli chromosome and probably specifies a new gene. Experiments to identify its gene product andcloning of the wildtype gene are in progress.
In three other fusion strains (ϕ1,2,5) β-galactosidase was induced two to three-fold in late logarithmic phase, in both a Δcya and a cya^+ background. Therefore these fusions are not to starvation genes, in a strict sense. However, they appear highly interesting, since in an otherwise wildtype background they confer a pleiotropic growth defect on maltose, glycerol, and ribose, which is suppressed by the addition of cAMP to the growth medium. ϕ1,2,5 do not map in or near cya or crp and therefore might be fusions to hitherto unidentified regulatory genes involved in catabolite repression.

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M. Laub and H.P. Jennissen

Tissue Distribution of Ubiquityl-Calmodulin Synthetase and Proteokinain in Rabbit

Recently we first demonstrated that a branched form of mammalian calmodulin (CaM) exists which is generated by the enzyme ubiquityl-calmodulin synthetase (uCaM-Synthetase, EC 6.3.?.?) of reticulocytes and cardiac muscle probably by isopeptide linkage of ubiquitin to calmodulin [1-3]. This branching reaction is strictly calcium dependent [1,2] and may be part of the degradation pathway of calmodulin [3]. However ubiquityl-calmodulin (uCaM) may also have as yet unknown biological functions, since it is still capable of binding Ca^{++} [1]. In cardiac muscle the classical ubiquitin-dependent proteolytic activity (see ref. [4]) could not be detected [5,6]. Instead, a ubiquitin-independent ATP-dependent protease which has been called proteokinain (m ca. 310 kDa) was found and partially purified from cardiac muscle [5,6]. In order to see if these two novel enzyme activities might be of major biological relevance their tissue distribution was studied in rabbit (for assays see [2,6]).

Ubiquityl-calmodulin synthetase in an activity range of 20-600 fkat/g wet weight was found in all tissues studied including: brain (cerebrum), erythrocytes, eye lens, kidney, liver, lung, smooth muscle (uterus), striated muscle (cardiac m.; diaphragm; red, slow twitch m.; white, fast twitch m.), reticulocytes, spleen & testis. Tissues with high enzyme contents e.g. reticulocytes, spleen & brain displayed crude extract activities of 10-15 fkat/mg protein. In controls, in the absence of added exogenous

calmodulin a significant ubiquitination of endogenous calmodulin (ca. 5 fkat/mg) in DEAE-cellulose enriched fractions e.g. of reticulocytes and testis was detected. This demonstrates that a spontaneous ubiquitination of endogenous tissue calmodulin occurs on sole addition of ATP/Mg^{++} and ^{125}I-ubiquitin. Studies of the time course of calmodulin ubiquitination over 120 minutes in several cases led to 'maximum curves' i.e. there was a significant decrease in generated uCaM after a certain time, indicating either a deconjugation or degradation of uCaM.

Similarly, ATP/Mg^{++}-dependent proteolytic activity compatible with proteokinain in an activity range of 5-20 arb. units/g wet weight was found in the crude extracts of the following tissues: brain (cerebrum), erythrocytes, eye lens, kidney, liver, lung, smooth muscle (uterus), striated muscle (cardiac m.; diaphragm; red, slow twitch m.; white, fast twitch m.), spleen & testis. After partial purification on DEAE-cellulose (elimination of endogenous ubiquitin) in no case could a stimulation of ATP/Mg^{++}-dependent proteolytic activity by exogenous ubiquitin be shown. The molecular mass of 300 kDa [4] and the detection of this enzyme activity in non-vascularized tissue such as the eye lens provide strong evidence that proteokinain activity is not due to contamination by α-macroglobulin/cathepsin complexes [7].

We conclude that the two tested enzymes belong to the normal repertoire of mammalian cells and therefore probably play an important role in their physiological function.

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Reinigung und immunhistochemische Charakterisierung von Calcium-bindenden Proteinen

Eine Gruppe von Calcium-bindenden Proteinen (CBP) mit scheinbarem Molekulargewicht von 65/67, 35 und 33 kD wurde aus Leber- und Morris Hepatom-Plasmamembranen nach Calcium-Komplexierung mit EDTA extrahiert, da diese Proteine über Calcium an die Plasmamembran gebunden sind (1).
In wenig differenziertem Morris Hepatom 7777-Gewebe kommen die kleinen CBP (35/33 kD) vermehrt vor im Gegensatz zu gut differenziertem Morris Hepatom 9121 oder Normallebergewebe.

Diese Proteine werden unter denaturierenden und nichtdenaturierenden Bedingungen durch selektive Extraktion vorgereinigt und isoliert.
Die weitere Reinigungsstufe und Trennung einzelner CBP erfolgte durch Kronenether-HPAC, Collagen-HPAC oder Fluor-Hydroxylapatit-HPLC. Durch diese HPLC-Methode lassen sich die CBP auch aus Organhomogenaten extrahieren.

Unter nicht-denaturierenden Bedingungen konnten Calcium-Bindungsmessungen durchgeführt werden. Außerdem konnte nachgewiesen werden, daß CBP-35 die Phospholipase-A_2-Aktivität hemmt.

Mittels immunhistochemischer Methoden konnten diese CBP in den polarisierten Epithelzellen, z.B. von Nierentubuli, Nebenhoden und Ausführungsgängen verschiedener Drüsen, gefunden werden
Außerdem war eine ausgeprägte Immunreaktivität in Plasmamembranen von Endothelzellen, Lymphozyten, Nervenzellen sowie im Cytoplasma von Muskelzellen nachweisbar.

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Native Polyacrylamide Gel Electrophoresis and Isoelectric Focusing as a New Analytical Tool for the Characterisation of Molecular Properties of Phospholipases

Acid phospholipases play an important role in the lysosomal catabolism of phospholipids. As yet there is very little known about the molecular properties of these enzymes, since none of them has been purified to homogeneity as yet. Lysosomal phospholipase A_1 (PLA1) has already been isolated in high purity from rat liver [1-4]. However, the molecular weights (MW) and isoelectric points (IEP) reported differ widely. We have now developed an analytical technique for assaying these molecular parameters even in relatively impure enzyme preparations of phospholipases A.

Isoelectric focusing was performed with 4% polyacrylamide rod gels, and native polyacrylamide gel electrophoresis (PAGE) either with 8% gels or with 10 - 30% gradient gels. After electrophoresis, these gels were sliced. The slices were then placed into a specific elution buffer and maintained for prolonged time at 4°C. Aliquots of the medium were subsequently analysed for lipolytic activity.

Employing this procedure, IEP and MW have been determined for acid PLA1 activities of rat liver lysosomes of different purities, and for acid PLA1 purified from rat liver Golgi vesicles. For purified lysosomal PLA1, an IEP of pH 4.9 was found which agrees well with the data described earlier [1]. Golgi PLA1 exhibited a major IEP of pH 5.7, and in addition two minor IEP values of pH 5.3 and 4.7 [5]. Consistent with our previous findings [3,4] and irrespective of the purity and mode of preparation, lysosomal PLA1 in native PAGE revealed two molecular masses, i.e. of 54 and 38 kDa. In these

experiments lysosomal PLA1 had been analysed in quite different preparations, i.e. in the soluble fraction of tritosomes [6], in lysosomes isolated by metrizamide gradient centrifugation [7] and in material purified from whole rat liver [4]. Our data now also clarify conflicting reports [1-4] on the MW of rat liver lysosomal PLA1.

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Kinetics of Aminoacylase I with N-Trifluoro-acetyl amino acids as Substrates

Trifluoro acetyl (TFA) amino acids as substrates of hog kidney aminoacylase I (EC 3.5.1.14) were first used by Fones and Lee [1] in order to investigate the stereospecifity of the enzyme. TFA amino acids contain a "labile" amide bond, which is hydrolyzed under mild conditions. In the present investigation we have compared the kinetic parameters of the aminoacylase-catalyzed hydrolysis of various TFA-AA with those of the corresponding acetyl-AA using a precise o-phtalaldehyd-based FIA method [2]. Unexpected results were obtained with Met-substrates, which show a lower k_{cat}-value for the labile substrate, which sheds light on the role of the Met side chain in transition state stabilization. To elucidate the possible conformations and charge distributions of the transition states of the respective substrate pairs, molecular mechanics calculations were performed [3]. We propose, that the side chain of Met substrates induces a local conformational change in the active site of the enzyme, which accounts for the observed deviation of the kinetics of these substrates.

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Effects of Bafilomycin on Endosomal pH and Pinocytosis in *Entamoeba histolytica*

Entamoeba histolytica is a primitive eukaryote that lacks a Golgi apparatus and a well-defined endoplasmic reticulum[1]. The amoebas exhibit a very high rate of pinocytosis, and pinocytic vesicles appear to perform most of the digestive functions that in higher cells are taken over by lysosomes[2].
From the fluorescence of pinocytized fluorescein isothiocyanate-dextran[3] we calculated the internal pH of the vesicles as 5.5±0.2; this value was largely independent of the external pH between pH 5 and 8. Bafilomycin A_1, a specific inhibitor of vacuolar (V-type) ATPases in higher cells[4], raised pinosomal pH (halfmaximal effect at 360 pmol/mg protein); concomitantly, it inhibited pinocytosis (50% inhibition at 90 pmol/mg protein).
These observations strongly suggest the presence of a V-type ATPase in pinocytic vesicles of *E. histolytica*. As in these cells plasma membrane and pinocytic membrane appear to be of identical composition[2], these observations also lend support to the notion[5] that early in evolution V-type ATPases resided on the plasma membrane.

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R. Lucius and R. Mentlein

Inactivation of the Neuropeptide Somatostatin by Brain Cells and Membranes

Apart from its role as a growth hormone - release inhibiting factor the tetradecapeptide somatostatin is

supposed to be a neurotransmitter or neuromodulator in the brain, especially in the cerebral cortex. Little is known about the inactivation of somatostatin. We have investigated the degradation of this peptide in cultures of dissociated neuronal and glial cells from rat cerebral cortex.

The different cell types were identified by immunohistochemistry for galactocerebroside (GC), glial fibrillary acidic protein (GFAP) and neuron-specific enolase (NSE). Incubations with ^{125}I-Tyr^{11}-somatostatin-14 were performed at 37°C in an incubation-buffer according to Horsthemke et al.[1]. Degradation was monitored by FPLC-separation of the products using a PepRPC HR 5/5 column (Pharmacia, Freiburg, BRD) and counting radioactivity in the eluted fractions.

When ^{125}I-Tyr^{11}-somatostatin-14 is added to the cultures, it is rapidly hydrolyzed by the cells. Somatostatin-degrading enzymes are present on neuronal and both oligodendroglial and astroglial cells. Degradation activity on somatostatin is higher in glial cultures than in neuronal cultures. The three cell types generate different degradation products indicating the presence of several somatostatin-degrading enzymes in rat brain. Various peptidase inhibitors were used to differentiate between individual proteases. In cultures of oligodendrocytes a bacitracin sensitive enzyme is almost entirely responsible for the degradation of somatostatin. Its activity can also be demonstrated on the myelin and microsomal subcellular fractions obtained from whole rat brain. The isolation and characterization of the different somatostatin-inactivation proteases is under investigation.

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Mathias Lübben and Günter Schäfer

F_0 - analogous proteolipid of the archaebacterium *Sulfolobus acidocaldarius*

Previously electron transport phosphorylation has been proposed to be the dominant metabolic pathway of energy conversion in aerobically grown cells of the thermoacidophilic archaebacterium *Sulfolobus acidocaldarius* [1]. By involving the proton as coupling ion the existence of F_0F_1-H^+-ATP synthase has been postulated from the N, N'-dicyclohexylcarbodiimide-sensitivity of ATP synthesis in intact cells [2]. From the data obtained for an ATPase, which is classified into the F_1 group with respect to its immunological and quaternary structural similarities[3,4], a molecular candidate for the terminal catalyst has been afforded. However, this F_1 - analogous ATPase barely carries the features of an extrinsic membrane protein and should *in vivo* be linked to the presumable membrane-embedded proton channel component F_0.

As a first approach to probe the existence of F_0, membranes labeled with [^{14}C] - N, N'-dicyclohexylcarbodiimide have been treated with chloroform/ methanol and the resulting proteolipid fraction has been analyzed by a specialized electrophoretic method[5]. Fluorographies of SDS-gels reveal a radioactive band at 6-7 kDa apparent molecular mass, as could be expected for an F_0-derived proteolipid. Moreover, probably due to its hydrophobic nature, it assembles to oligomers of 14 kDa (n=2) and higher association numbers.

This tendency of forming adducts is even enhanced during further purification of the proteolipid by CM-cellulose and Sephadex LH-60 chromatography, at least indicating that this property results from a single type of polypeptide.

Because the proteolipid is dominantly composed of apolar amino acids, an extremely low polarity index of 18.2 % is calculated, which is consistent with data obtained for F_0 proteolipids from eubacteria and eukaryotic organelles.

Strong indications on the qualification as F_0-analogous proteolipid result from amino acid sequences from the N-terminus and from cyanogen bromide fragments. By comparing the partial sequence of the *Sulfolobus* proteolipid (33 residues) with those of eubacterial, chloroplast and mitochondrial sources, a number of invariant and conservatively substituted amino acids are assigned. These are especially clustered in the membrane spanning part of the polypeptide chain, as defined from secondary structure predictions of subunit c from *E. coli*.

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Chloride as cofactor of photosynthetic water oxidation

Photosystem II is a complex of at least 15 polypeptides including the photoreaction centre and a manganese containing catalytic centre of the water oxidizing enzyme. Ca^{2+} and Cl^- serve as cofactors for catalysis of dioxygen formation. We reinvestigated the role of Cl^-. Cl^- is essential, but according to measurements of the extended X-ray absorption fine structure (EXAFS) of the catalytic centre (Yachandra et al., 1986), it is not detectable as ligand of EPR-active manganese.
We measured oxygen evolution under continuous illumination, proton release and electrochromic absorption changes under single flash excitation of dark adapted thylakoids. These are indicators of the various activities (photochemical charge separation, catalysis of water oxidation) of the photosystem II complex.
We found that Cl^--deficiency inhibits the S_2-S_3 transition out of the four-stepped oxidation cycle from 2 H_2O to O_2. This is in agreement with previous EPR results by Ono et al. (1987).
Further inquiries are directed to the question, whether chloride takes part in chemical reactions or if chloride displacements are required for the electrostatic balance of the manganese centre from which four electrons are abstracted during water oxidation. In this respect it is noteworthy, that addition of a known blocker of Cl^--channels in various tissues, mimicked Cl^--deficiency in the oxygen evolution rates and in the pattern of charge separation, even in the presence of chloride.

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The Structure of the frozen hydrated ATP Synthase Complex (ECF_1F_0) of Escherichia Coli from Electron Microscopy

The structural realtionship of the catalytic portion (ECF_1) of the E.coli F_1F_0 ATP synthase (ECF_1F_0, EC 3.6.1.3.) to the intact, membrane-bound complex has been determined by cryoelectron microscopy and image analysis of single, unordered (though oriented) particles. ECF_1F_0, reconstituted into membrane structures, has been preserved and examined in its native state in a layer of amorphous ice. Side views of the ECF_1F_0 show the same elongated bilobed and trilobed projection of the ECF_1 views, shown previously (1) to be normal to the hexagonal projection. The elongated aqueous cavity of the ECF_1 is perpendicular to the membrane bilayer profile in the bilobed view. ECF_1 is separated from the membrane embedded F_0 by a narrow stalk ~40 Å long and ~25-30 Å thick. The F_0 part extends from the lipid bilayer by ~10 Å on the side facing the ECF_1. There is no clear extension of the protein on the opposite side of the membrane. The comparison with the frozen-hydrated structure of the chloroplast ATP synthase and the assignment of nucleotide binding sites (2), and its significance to an indirect coupling mechanism is discussed.

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"Autoinhibition" of Ap_3A-induced platelet aggregation by Ap_3A

The dinucleotides adenosine(5')triphospho(5')adenosine (Ap_3A) and adenosine(5')tetraphospho(5')adenosine (Ap_4A) are stored in high amounts in the dense granules of human platelets and are released upon activation of thrombocytes(1,2). Extracellular dinucleotides influence platelet aggregation in an antagonistic way: Ap_3A induces aggregation whereas Ap_4A inhibits this process(3). Ap_3A-induced aggregation is essentially dependent on the presence of a plasma enzyme splitting the dinucleotide into AMP and the well known platelet agonist ADP(4).

We have studied the concentration dependence of the Ap_3A effect. Surprisingly, Ap_3A exhibited an autoinhibition at higher concentrations, a bell-shaped dose-response curve was obtained. Aggregation was maximal with 4 µmol/l Ap_3A, with higher concentrations the responses decreased. Since ADP did not produce such a bell-shaped curve, it was hypothesized that the autoinhibition was due to Ap_3A which had not been degraded to ADP and which competitively interfered with ADP at the platelet receptor.

This suggestion was supported by experiments measuring ADP-induced platelet aggregation (concentrations: 0.5 and 400 µmol/l) in the presence or absence of Ap_3A, Ap_4A or ATP (20 to 200 µmol/l). All three nucleotides caused a parallel right-shift of the dose-response curve, indicative for a competitive mechanism. Ap_3A was a weak inhibitor of ADP-induced aggregation (K_i = 400 µmol/l). For comparison, Ap_4A and ATP were potent inhibitors (K_i = 27 and 15 µmol/l, respectively). Besides explaining the autoinhibition by Ap_3A, these results add strong experimental evidence to the view of Ap_4A being a competitive inhibitor of ADP-induced aggregation, which was hitherto only deduced from structural similarities.

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H. Luppa, G. Poeggel and H. Brandt

Immunohistochemical Localization of Protein kinase A in the Rat Hippocampus

Histochemical enzyme localization is generally possible by enzymehistochemical and immunohistochemical methods. We decided on the way of the immunohistochemical detection because the substrat, necessary for the enzymehistochemical localization is hydrolysed also by several other enzymes.
The catalytic subunit of beef heart protein kinase A was used as antigen for the production of antibodies against protein kinase A. With the aid of the immunoblotting-technique, a cross reaction could be detected between the derived antibody and the protein kinase A from rat brain. For the immunohistochemical detection of the antigen we used the PAP-technique of Sternberger. The brain tissue was fixed with 4 % paraformaldehyde, resulting in a good antigen stabilization. For electron microscopy 0.25 % glutaraldehyde was added to the paraformaldehyde.
A positive reaction could be observed in cytoplasm and many nuclei of pyramidal cells and their basal dendrites, in glial cells and capillaries. Concerning the subcellular localization, reaction product could be observed in microtubules, Golgi-apparatus, endoplasmic reticulum, nuclear envelope, chromatin and on the surface of mitochondrias.

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The OmpA protein as a model for export incompatibility in Escherichia coli

It has been concluded that the mature part of the precursor to the outer membrane protein OmpA contains no information which is essential to translocation across the inner membrane (1-3). We have shown that although such essential 'positive' information appears to be lacking, certain types of sequences are clearly incompatible with export. i) An internal stretch of 16, but not 12 or less, hydrophobic residues resulted in abortive translocation as the lipophilic sequence anchored the polypeptide in the inner membrane in the 'head on' orientation or functioned as a signal-anchor sequence. ii) Basic residues at the amino terminus of the mature polypeptide dramatically decreased the efficiency of export, most likely due to interference with the function of the signal sequence. iii) Three different non-secretory polypeptides (a phage tail fiber fragment, mouse dihydrofolate reductase and E. coli β-galactosidase) could be translocated across the E. coli inner membrane when fused to pro-OmpA, but a general block in export occurred when synthesis of the latter two hybrids was not maintained at low levels. iv) A lower size limit was observed for export of pro-OmpA fragments. Thus, exported proteins, in general, have presumably evolved in a way avoiding/or minimizing the presence of these types of sequence.

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T. Mair, M. Höfer and E. Gießler-Andersen

Reconstituted Plasma Membrane Vesicles from Schizosaccharomyces pombe: Generation of $\Delta\mu_{H^+}$ and Sugar Transport

Reconstitution of plasma membranes from the yeast Schizosaccharomyces pombe with phospholipids by means of a detergent-dialysis procedure yielded sealed vesicles (PMV). Upon addition of Mg-ATP the reconstituted PMV generated an electrochemical proton gradient ($\Delta\mu_{H^+}$), inside acidic and positive (1). The reconstituted PMV displayed a low unspecific cation permeability as demonstrated for K^+ by manyfold acceleration of ΔpH formation by the ionophore valinomycin, and for H^+ by slow dissipation of preformed ΔpH following

removal of Mg-ATP by addition of hexokinase + glucose to PMV.

For sugar transport studies PMV were separated from medium by short low-speed centrifugation through a Sephadex-G50 gel column. PMV in each experiment were quantified by determination of protein content. The uptake of monosaccharides was time dependent, reaching a steady state within 15 minutes. D-glucose and 2-deoxy-D-glucose competed for the uptake. In the presence of ATP ($\Delta\mu_{H^+}$ directed from the inside of PMV to the outside) considerably less 2-DOG was taken up by PMV due to oppositely proceeding H^+/2-DOG symport; addition of an uncoupler led to increased influx of 2-DOG into PMV. ATP added to PMV preloaded with 2-DOG caused an outflow of the sugar. The gradient of 2-DOG maintained was about two fold.

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Fatty Acid Acylation of Human Erythrocyte Membrane Proteins: Evidence for a Spectrin Fraction which is Tightly Associated with the Membrane

Fatty acid acylation of membrane proteins was studied in intact human erythrocytes incubated with 9,10(n)-^{3}H-palmitic acid. A polypeptide of 55 kDa in the band 4.6 region of electropherogrammes was rapidly labeled and reached saturation within one hour at 37°C and 3.5 µM ^{3}H-palmitate. Labeling of most other polypeptides (band 4.1 and 4.9 and bands at 40 and 30 kDa) did not reach saturation within 6 hours. The incorporated label was proportional to the specific radioactivity of ^{3}H-palmitate and could be chased, indicating a reversible palmitoylation. Palmitate incorporation into high molecular weight skeletal proteins, which were the least labeled, was studied by extracting spectrin, ankyrin and an inside-out vesicle (IOV)-associated form of spectrin(1). IOV retain about 5 % of the total spectrin. This spectrin remained vesicle-associated after extraction of ankyrin with 1 M KCl and was labeled with ^{3}H-palmitate. Low ionic strength extractable spectrin as well as ankyrin did not contain appreciable amounts of label after 12 hours of incubation of intact cells. The selective labeling of a minute fraction of cellular spectrin suggests the existence of a fatty acid acylated form of spectrin, which is tightly associated with the membrane. The amount of membrane-associated spectrin can be enhanced by a cAMP-dependent phosphorylation(1). The tightly membrane-associated spectrin could be solubilized by 4 M urea in low ionic strength buffer. In its purified form (gel filtration; ion exchange chromatography) it retained incorporated ^{3}H-palmitate and contained less than 0.1 mol ^{3}H-palmitate/mol, when recovered from cells incubated for 12 hours. This reflects a very low turnover of the fatty acid moiety in the membrane-associated spectrin.

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Repetitive Elements Associated with tRNA Genes in *Dictyostelium discoideum*

Two repetitive elements occur in strict association with tRNA genes in the cellular slime mold *Dictyostelium discoideum*. One element, termed DRE (*Dictyostelium* repetitive element), is always found approximately 50 bp upstream of various tRNA genes. This element is about 6 kb in length and is characterized by all typical features of a retrotransposon. The element is flanked by long terminal direct repeats and putative products of open reading frames show significant similarities to reverse transcriptase and *env* gene products.

The second repetitive element (Tdd3) always occurs approximately 100 nucleotides downstream from a variety of different tRNA genes. In strain AX-2 about 200 DRE elements and 50 Tdd3 elements are present. This strict position specific association of DRE and Tdd3 with *Dictyostelium* tRNA genes suggests a new function of tRNA genes in this organism: tRNA genes may act as genomic landmarks for the integration of mobile genetic elements.

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Ulrich Matzner and Klaus Scheller

Steroid Hormones Control the Stage Specific Transport of Arylphorin from Haemolymph into Fat Body

Arylphorin, a group of multifunctional larval serum protein in insect larvae is encoded by a multigene family which is stage- and tissue specifically expressed(1). The protein, consisting of six 80 kD-subunits, is synthesized by the fat body and secreted into the haemolymph from where it is reabsorbed by the fat bodies of larvae which are ready for metamorphosis. The reabsorption of arylphorin is paralleled by a rapid increase in the ecdysteroid titre. Injections of 20-OH-ecdysone as well as addition of physiological concentrations of the

hormone to the incubation medium where the fat bodies were kept in vitro, induced an incorporation of radioactively labelled arylphorin in the fat body cells.
We report experiments which demonstrate that arylphorin is bound to fat body membranes, suggesting the presence of a receptor which becomes activated by ecdysteroids. We describe our exertions to characterize the arylphorin receptor by immunological techniques. The mode of action how steroids activate a receptor protein to bind a specific protein will be discussed.

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Beate Meier

Comparative Studies on a Superoxide Dismutase Exhibiting Enzymatic Activity with Iron or Manganese as Active Metal Cofactor

Propionibacterium freudenreichii sp. shermanii produces a single superoxide dismutase which contains either iron or manganese as the active metal cofactor, depending on the metal supply to the culture medium.

The secondary structure of the in vivo and in vitro iron to manganese exchanged superoxide dismutase was studied by CD-spectroscopy. Both enzymes exhibited a β-structure and changed to α-structure by addition of μmolar concentrations of azide or fluoride. The tryptophan region, studied by CD-, fluorescence- and UV-spectroscopy, was not altered by addition of these anions.

The Mn-SOD was less stable towards high temperatures, acid and alkaline pH, and denaturating agents than the iron enzyme. Azide reversibly inhibited the Fe-SOD to 30% at a concentration of 10 mmol/l, but caused no inhibition of the Mn-SOD. Higher concentrations of azide up to 100 mmol/l did not alter the remaining activity. Both enzymes were insensitive to cyanide. Hydrogenperoxide destroied the activity of both enzymes. The protein moiety of the Fe- and Mn-SOD could be degradated by trypsin to a single fragment without any effects on the enzymatic activities.

Both enzymes show identical fused precipitation lines in an immunodiffusion test.

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Mutants of Saccharomyces cerevisiae Impaired in Peroxisome Biogenesis

Peroxisomes are ubiquitous in eukaryotic organisms including fungi and fulfil a number of essential functions in these cells. There is accumulating evidence that peroxisomes proliferate by growth and division like mitochondria and chloroplasts. It has recently been discovered that peroxisome proliferation can also be induced in S. cerevisiae[1]. This finding together with the well developed classical and molecular genetics of this yeast provides a possibility to dissect the mechanism of peroxisome biogenesis by a combined biochemical and genetic approach.

A recently described screening procedure[2] for peroxisomal mutants was based on the observation that intact peroxisomes are necessary for growth on oleate. The resulting oleate-non-utilizing mutants were subsequently analysed by determination of peroxisomal enzyme activities and by electron microscopy. This protocol led to the detection of peroxisome-deficient yeast mutants (pas mutants) which like Zellweger fibroblasts frequently show a cytosolic localization of peroxisomal enzymes.

Therefore a modified screening protocol included the determination of the subcellular localization of peroxisomal enzymes by means of differential centrifugation. This led to the discovery of seven additional mutants impaired in peroxisome biogenesis. Two of them seem to be temperature sensitive. Genetic analysis of the new mutants together with the original four pas mutants showed that they fell into at least six different complementation groups.

Peroxisomal mutants of S. cerevisiae exhibit great phenotypical similarities to cells of patients with peroxisomal diseases. Thus, the possibility of using these yeast mutants as an experimental model system will be discussed.

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W.E. Merz, P. Harbarth and P. Licht

Evidence for a regulation of human choriogonadotropin synthesis by gonadoliberin

The cytotrophoblast of human placenta synthezises a number of peptide hormones (gonadoliberin (GnRH), corticoliberin, somatostatin) which are familiar also as hypothalamic products whereas the syncytiotrophoblast produces tropic hormones like human choriogonadotropin (hCG), corticotropin, and placenta lactogen. We have investigated the hypothesis whether GnRH stimulates hCG biosynthesis in analogy to the effect of GnRH on the gonadotropin synthesis by the pituitary.
Human placenta tissue (8.-10. week of gestation) was continuously perifused (3 ml/h) with medium 199 and treated with 2 GnRH pulses (1 nM-10 μM; 30 min) at 24 h and 36 h (in some cases 48 h) after beginning of culture. Three different effects on the hCG secretion were observed: 1) All tissue pieces treated with a GnRH pulse (n=4-5 per experiment) responded with a significant transient increase of the hCG secretion rate. 2) GnRH pulses caused a twofold increased hCG secretion rate. This effect was evident several hours after the second GnRH pulse and was maintained for at least 24 h. It was probably due to a direct effect of GnRH on gene activity since the α-subunit mRNA level at day 3 of GnRH treated cultures was also increased twofold as shown in dot blots as well as Northern blots using ^{32}P-labeled cDNA. Based on the α-subunit mRNA levels an optimum of the GnRH effect was observed at a concentration of 100 nM. 3) In superfusion culture placenta tissue showed an episodic secretion of hCG with a frequency of $(210\text{-}270\ min)^{-1}$. GnRH caused a significant increase of the amplitude as well as of the frequency. In conclusion, exogenous GnRH caused a complex response of first trimester placenta tissue with respect to hCG biosynthesis and secretion. This supports suggestions that GnRH produced by the cytotrophoblast regulates the production of hCG in the syncytiotrophoblast. So far, a functional analogy between these two cell types of the placenta and the hypothalamic-pituitary axis might exist.

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Effect of Cytokines and Liver Fat Storing Cell Conditioned Medium on Heparan Sulfate and DNA Synthesis of Hepatocytes in Culture

Liver cell regeneration is controlled by a great number of factors. Some of these are known (TGFalpha, TGFß), but their cellular sources are not clearly established. Heparan sulfate (HS) is the almost exclusive glycosaminoglycan (GAG) synthesized by hepatocytes (PC) of normal liver and was proposed to be involved in regulation of proliferation (1). The factors influencing the synthesis of HS and other PGs are still obscure.
We studied the effect of the cytokines TGFalpha, TGFß, EGF and (as a potential source) fat storing cell (FSC) conditioned medium on the synthesis of total sulfated GAG, heparan sulfate, and DNA in cultured PC, isolated from normal and regenerating rat liver. PC were isolated according to Seglen, tested for impurities by desmin and vimentin immuno staining, and kept in culture up to 3 days in Hams F-12 medium, containing or missing 5% fetal calf serum (FCS), 0.02 U insulin/ml and 0.2% BSA. Various dilutions of dialysed (3500 MW cut off), native or short time acidified and reneutralized conditioned medium from secondary cultures of FSC were added 2 h after seeding for 40 h to PC cultures and DNA content, (^{3}H)-thymidine and bromodeoxyuridine incorporation for the last 24 h, and the production of (^{35}S)-labeled GAGs and HS were measured.
Results indicate, that EGF and TGFalpha strongly stimulate the proliferation of hepatocytes in culture; (^{3}H)-thymidine incorporation into DNA of normal PC was enhanced 3 - 4 fold. TGFß reduced the EGF, TGFalpha or FCS stimulated (^{3}H)thymidine incorporation to nearly the level of unstimulated PC. FSC conditioned media showed a strong inhibitory effect on DNA synthesis of PC. The incorporation of (^{3}H)-thymidine into DNA of normal PC was reduced by 30% and 20% with 1:2 and 1:4 dilutions of native FSC conditioned medium. Using shortly acidified (20 min, pH 2.0) FSC medium the inhibitory effect was increased up to 60%. In PC cultures from regenerating liver 1:2 and 1:4 dilutions of FSC medium inhibited (^{3}H)-thymidine incorporation by about 70%. Again native FSC medium was less potent (50% inhibition at 1:2 dilution). FSC medium counteracted the TGFalpha induced stimulation of PC DNA synthesis very efficiently: 65% inhibition at 1:2 and 1:4 dilutions of acidified FSC medium. In binding studies of ^{125}I-TGFß on receptors of PC it was shown, that acidified FSC medium can displace TGFß from its receptors on PC. Much of the inhibitory activity of FSC medium can be abolished by neutralizing polyclonal antibody against human TGFß$_1$. Neither FSC conditioned medium, nor any of the tested cytokines showed an effect on hepatocellular GAG or HS synthesis.
We conclude, that FSC secrete a DNA synthesis inhibitory activity into the medium, which is TGFß, since it is activated by short acidification, can compete with ^{125}I-TGFß in the radio receptor assay, and is neutralized by specific antibodies. In situ, FSC might control PC replication in injured liver by a paracrine mechanism. Modulation of HS synthesis seems to be not involved in the changes of DNA synthesis of PC.

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Wolfgang Meyer and Günter Schäfer

H^+/O-stoichiometries of the thermoacidophilic archaebacterium *Sulfolobus acidocaldarius*

Earlier experiments with whole cells showed that in *Sulfolobus acidocaldarius* the flow of electrons through the respiratory chain is coupled to the extrusion of protons (1). The electrochemical membrane potential is composed of a large pH-gradient and a small inside negative membrane potential at an external pH of 3.5 and 50-60 °C (2). The earlier experiments on actual proton extrusion were performed in a medium containing high ammonium concentrations in accordance with the optimum growth medium reported by Brock (3). Here we show that ammonium largely modifies the H^+/O ratio.
Oxygen pulses were given into anaerobic cell suspensions and the initial rates of proton extrusion and respiration were registered simultaneously with a pH- and a clark-type oxygen electrode. In another set of experiments initial proton extrusion was measured spectrophotometrically in a stopped-flow device. Under all applied conditions the initial respiratory rate was identical (25-30 nmol O_2/min · mg), while the rate of proton extrusion varied considerably. In

presence of 9.8 mM ammonium (as under usual growth conditions) a H^+/O-stoichiometry of 0.3 was measured. Addition of K^+ (20mM) and valinomycin (4.2 nmol/mg) increased the H^+/O ratio to 1.3. In absence of ammonium however the basic H^+/O was 4, with 10 mM K^+ present, even in absence of valinomycin. Stopped-flow measurements revealed a biphasic H^+ extrusion with a short and very fast initial phase. During this transitory phase H^+/O ratios of 6-8 could be calculated (based on the above given respiratory rate), which could be pushed even higher by valinomycin.
These results clearly demonstrate the coupling of potassium fluxes to the proton extrusion. An inhibitory effect of ammonium on the potassium fluxes might be possible. Further experiments will reveal the molecular relationship between the potassium and the proton transport systems.

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^{14}C-Mg-ADP and 2-Azido-(β, γ ^{32}P)-ATP binding to solubilized membrane ATPase of Sulfolobus acidocaldarius

Sulfolobus acidocaldarius is an aerobically growing thermo-acidophilic archaebacterium. From its membrane an ATPase has been isolated [1] which is considered to represent the catalytic part of an F_1-analogous H^+-conducting ATP-synthase.
The present study is addressing the question, whether or not the characteristics of nucleotide binding to the archaebacterial ATPase from Sulfolobus acidocaldarius are resembling those from eubacterial and eukaryotic F-type ATPases.

Binding studies of Mg^{2+}-^{14}C-ADP to Sulfolobus ATPase were performed by equilibrium dialysis at 70°C. At least 3 mol nucleotide/mol ATPase could be titrated, a figure which fits excellently the oligomeric structure proposed to contain 3 parts of the major subunits α and β [1]. Due to the scattering of data at high nucleotide concentrations it could not be excluded that additional sites of lower affinity are present.
The enzymatic activity of the Sulfolobus acidocaldarius ATPase with the substrates Mg-ATP and Mg-2-Azido-ATP (synthesized according to Melese et al. [2]) was determined at 70 °C in the dark (nucleotide/Mg^{2+}-ratio = 4). A K_M-value of 200 µM for ATP and 400 µM for 2-Azido-ATP and also similar values of v_{max} demonstrating this analogue to be a comparably efficient substrate.
2-Azido-ADP acts as an inhibitor of ATPase activity. Preincubation of ATPase with 2-Azido-ADP at 70°C in the dark and subsequent photolysis at 254 nm caused 42% inhibition of the enzymatic activity, 18 % inhibition was observed under the same conditions without irradation. This suggests that 2-azido-analogues are suitable photoaffinity probes for covalent tagging of the nucleotide binding sites. Covalent labeling of the ATPase with 2-Azido-(β, γ ^{32}P)-ATP/EDTA under saturating conditions was performed by preincubation at 70 °C of ATPase with the nucleotide, seperation of the enzyme by centrifuged column [3] followed by photolysis at 256 nm. Analysis of the products by SDS-PAGE resulted in a labeling of both, the β- and *mainly* the α-subunit of Sulfolobus acidocaldarius. Labeling is partially prevented by addition of cold ADP after the preincubation.

The ATPase of Sulfolobus acidocaldarius is reminiscent of the F-type ATPases, for example: immunological properties of the β-subunit, molecular mass, quaternary structure of the soluble part of the enzyme [1], presence of a 6-7 kDa DCCD-sensitive proteolipid in the membrane[4], and at least 3 reversible nucleotide binding sites. Reminiscence of V-type ATPase is also obvious in respect to: inhibitor sensitivity, immunological properties [1], sequence homologies [5] and labeling mainly of the α-subunit with 2-Azido-ATP. These results underline the chimeric nature of this enzyme, emphasizing the close evolutionary relation between F-type and V-type ATPases.

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Copper(I)-thionein and Inflammation

Metallothioneins belong to a class of ubiquitous proteins (M_r = 6 kD). They are characterized by an unusual high content of cysteine (> 30 mol %) and can be induced by transition metals, stress related hormones, interferons and bacterial endotoxins [1]. Their physiological role is not known. Copper, Zinc- and cysteine storage and transport functions are in debate. In the course of inflammatory processes plasma copper levels can increase up to 300% [2]. It was therefore of interest to investigate the participation of Cu-thionein in inflammatory disorders. The reactivity of copper(I)-thionein, isolated from S. cerevisiae on tetra-o-decanoyl-phorbol-13-acetate activated polymorph-nuclear leucocytes was evaluated in unseparated human blood [3]. 18 µM thionein-Cu was sufficient to inhibit the oxidative burst dependent superoxide production of human blood phagocytes by 50%. The galactosamine/endotoxin induced hepatitis in mice seemed most appropriate to monitor the antiinflammatory reactivy of copper-thionein in vivo. Upon intraperitoneal application of 32.5 µmolal thionein-Cu the release of sorbitol dehydrogenase and

superoxide dismutase was restricted to 55%. The levels of both enzymes are increased during hepatocellular injuries. No Cu_2Zn_2superoxide dismutase in the serum at all was detected when 65 µmolal thionein-Cu was administered. The in vitro simulation of an oxidative stress dependent flux of activated oxygen species caused by the aqueous decay of K_3CrO_8 to $\cdot O_2^-$, $^1gO_2$, $\cdot OH$ and H_2O_2 (4) was inhibited by 50% in the presence of 78 µM thionein-Cu. Furthermore the singlet oxygen dependent NADPH oxidation caused by UVA illuminated psoralen was considerably diminished.

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Enzymatic characterization of cultured endothelial cells from cerebral capillaries

The endothelial cells of cerebral capillaries form a selective barrier between blood and brain. Attempts have been made to employ an *in vitro* model that possesses the characteristics typical for this so-called blood-brain barrier. We have successfully cultured capillary endothelial cells derived from porcine brain by pure enzymatic digestion. Primary cultures have been characterized by several enzymatic properties. Freshly isolated cells contain high level of γ-glutamyl transpeptidase and alkaline phosphatase. The enzyme activity level decreases with time in culture, which is correlated with the increasing number of newly formed cells. Different surface conditions (collagen, laminin, fibronectin, basement membrane) did not influence the enzyme activity decrease. In the absence of serum in the medium, which causes an inhibition of the cell proliferation, the enzyme activity with respect to cell protein remains constant on a high level. We conclude that typical blood-brain barrier markers are only present in freshly isolated cells. Marker enzyme activity is not expressed in newly formed cells which explains the activity decrease with time in culture. This is different for a common endothelial cell marker, the angiotensin converting enzyme, which remains present in cells that proliferate in culture.

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Membrane-bound succinate dehydrogenase activity in the thermoacidophilic archaebacterium Sulfolobus acidocaldarius

In cells of Sulfolobus acidocaldarius (DSM 639) -grown aerobically on a glutamate/potassium sulfate containing Brock's medium (1) at T = 80°C, pH 2.5- substantial SDH-activity is present in the cytosolic supernatant after cell disruption with ultrasonification, Ribi- or French press (2). Only 10 to 30% of the total activity -varying between preparations- can be found in the membrane fraction. Sodium pyrophosphate at pH 8.5 extracts this activity from the membrane, so that SDH can be detected in the resulting supernatant as a succinate-PMS-DCPIP reductase. Further purification of the pyrophosphate supernatant by DEAE-Sepharose anion exchange at pH 8.0, gelfiltration and CM-Sepharose cation exchange at pH 6.4 and T = 4°C leads to a partially purified enzyme with a specific activity of 3 to 4 U/mg in the PMS-DCPIP assay at T = 55°C and pH 6.5. It shows fluorescence excitation and emission spectra characteristic for histidyl-bound flavin. Isoelectric focussing with ampholines reveals activity stainable protein bands between pH 6.5 and 7. This preparation on SDS-PAGE shows only one polypeptide subunit in the molecular weight range of 66 kDa, whereas smaller subunits as reported for mitochondrial and eubacterial SDH-complexes (for review see (3)) are lacking. The purified SDH is inhibited by malonate in a competitive manner.
Sulfolobus membranes display succinate oxidase activity, which is inhibited by malonate and cyanide and is gradually lost after pyrophosphate treatment. Electron flow occurs via the cytochromes in the membrane. Cytochrome reduction measured by dual wavelength spectroscopy at (440-418)nm after blocking terminal oxidases (4) with cyanide reveals first order kinetics; the rate of the cytochrome reduction depends on the SDH-activity present on the membrane. Although the results suggest electron transport from succinate reducible flavoprotein to terminal cyanide-sensitive oxidases little is known so far about intermediate redox systems in this archaebacterial respiratory chain. As such caldariella quinone (5) may be considered, which recently has been shown to deliver electrons to an aa_3-type oxidase (4).

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Toward a Mechanism for the Electric Permeabilisation of Lipid Bilayer Vesicles - Model for Membrane Electroporation

The transient permeabilisation of biological membranes by high voltage pulses, discovered in 1972 (1), is the fundamental experimental basis of cell electrotransformation and of cell electrofusion (2).

Evidence is accumulating that it is primarily the lipid part of biological membranes that is permeabilized and is at the same time fusogenic. Using lipid bilayer vesicles as a model, electrooptic and conductometric relaxations in high electric fields indicate electric pore formation. The linear dichroism data suggest that, above a threshold value of the external electric field, the lipids and optically anisotropic membrane markers change the orientation within the pore wall and are transiently more exposed to the aqueous environment. The relaxation kinetic data are consistently interpreted in terms of structural transitions, from small hydrophobic pores or defect sites to larger hydrophilic electropores or electrocracks (3).

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S. Müller, R.D. Walter

Polyamine Oxidase - The Regulatory Enzyme of Nematodic Polyamine Metabolism

Polyamines are essential for all organisms as they are involved in proliferation and division of cells. Polyamine synthesis and degradation are regulated in mammals by ornithine decarboxylase, S-adenosylmethionine decarboxylase and polyamine N-acetyltransferase, respectively. In parasitic nematodes interconversion and distribution pattern of polyamines are regulated by polyamine oxidase. These parasites lack ornithine decarboxylase - the initial enzyme in polyamine biosynthesis - consequently they depend on polyamine uptake from their host. Polyamine N -acetyltransferase, the rate limiting step in the reversed pathway and degradation could not be demonstrated in nematodes. In agreement with this observation, the nematodic polyamine oxidase catalyzes the oxidative deamination of spermine and spermidine resulting in aminopropionaldehyde, H_2O_2 and spermidine/putrescine, as identified by HPLC. The enzyme is active toward spermine, spermidine, nor-spermine, nor-spermidine, tryptamine and benzylamine, with K_m values of 0.35 mM, 0.66 mM, 2 mM, 5 mM, 2.5 mM and 1.6 mM, respectively. Activity of polyamine oxidase depends on SH-groups and the inhibition of enzyme activity by carbonyl reagents demonstrates its dependance on a cofactor such as pyrridoxalphosphat. The polyamine oxidase of Ascaris suum was partially purified.The molecular weight determined by gelfiltration is about 80 000. The isoelectric point was found to be pH 7.2 as determined by preparative isoelectric focusing.

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Reconstituted Proteoliposomes from Bacterial ATP-Synthase and Monomeric Bacteriorhodopsin

The enzymatic reaction of ATP-synthase (EC3.6.1.34) as well as its regulation are suggested to be accompanied by structural changes of this integral membrane protein and triggered by the electrochemical proton potential difference of the membranes. This is in vivo generated by the electron transfer chains and can in vitro be produced by i) mixing techniques, ii) flash photolysis of caged acids[1] or iii) additional membrane proteins, e.g. monomeric bacteriorhodopsin[2-5].
ATP-synthase from Rhodospirillum rubrum FR1 and bacteriorhodopsin from Halobacterium halobium were isolated from fresh grown bacteria and purified by gel chromatography. Monomeric bacteriorhodopsin (mBR) was prepared by treatment of

purple membranes with Triton-X-100, which was removed during the subsequent HPLC run[2,3]. ATP-synthase and mBR were reconstituted each or together into preformed small unilamellar lipid vesicles in the presence of detergents (bile acids). As shown by neutron scattering[6], the liposomes are impregnated by the detergent during the reconstitution, but not destroyed. Starting with sonified vesicles of 25 nm diameter, the finally obtained proteoliposomes exhibit a size of 48 nm in case of reconstitution of ATP-synthase[7,8] and 80-90 nm in case of co-reconstitution of ATP-synthase and mBR[4], according to freeze fracture electron microscopy. For some experiments the crude reconstituate was subfractionated by density gradient ultracentrifugation or gel chromatography. Protonic coupling of ATP-synthase and proton fluxes in reconstituted proteoliposomes were demonstrated by light driven ATP-synthesis, uncoupler (FCCP) stimulation of ATP-hydrolysis and by estimation of the light absorption of pH-indicators entrapped in the lumen of the liposomes.

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A rapid non-radioactive HPLC-with-column-switching assay for identification of proteins involved in the inositol phosphate metabolism

Picomol amounts of inositol phosphates can be separated and quantified by HPLC using the recently developed 'metal-dye-detection'[1]. In order to improve the analysis frequency of such a fully automated HPLC system, column switching technique was developed in which one column is loaded while the other one is eluted. Cycle times of less than 8 minutes where thus achieved for simple inositol phosphate isomer resolution applications. For example, enzyme activities of inositol phosphate metabolizing enzymes such as kinases and phosphatases can thus be easily determined by separating substrate and product of a non radioactive assay mixture within a few minutes. Likewise, gel filtration experiments according to Hummel and Dryer[2] designed to identify possible inositol phosphate binding proteins can be realized nonradioactively by analyzing the changes in saturation of such columns, equilibrated with one (or more) inositol phosphate isomers of interest.

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Kinetics of Phosphate Formation by Bacterial F_1ATPase during one Enzymatic Turnover

In F_1ATPase (EC 3.6.1.34) three substrate binding sites were found to be involved in the enzymatic cleavage of ATP[1]. There are several models to discribe the interaction of these catalytic sites. A matter still under discussion is the question of functional and structural asymmetry in the proteincomplex[2].
For the investigation of the time coarse of phosphate libration it is necessary to synchronize the F_1-molecules i.e. the main part of the protein molecules start to turn over within a time interval which is much shorter than the reaction cycle. Under these conditions a synchronous and measurable phosphate libration is expected.
The hydrolytic activity of F_1ATPase from Micrococcus luteus can be slowed down by decreasing temperature, due to the high activation energy[3]. At 13°C we found F_1ATPase of M. luteus to be synchronizable by rapid mixing the protein solution with millimolar amounts of substrate (CaATP). Time resolved phosphate analysis of acid quenched aliquotes taken from the reaction mixture revealed a discontinous phosphate formation. Periodically after a short phosphate libration a plateau occurs. Within a 18 second period (one turnover) there is no phosphate production measurable for about 5 to 9 seconds. The distance between two plateaus is two moles phosphate per mol F_1ATPase. In one experiment up to four plateaus were detected. These results support models for the hydrolysis mechanism of F_1ATPase with only

two sites acting cooperativly, the third site being inactive or less active.

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Catenin, a Protein Strongly Associated with the Intermediate Filament

We were able to isolate a cDNA clone (p550) which is transcribed from a mRNA species decreasing in amount during embryogenesis and prolonged cell culture. The gene product p550 (=Catenin) was identified using specific antibodies directed against a genetically manipulated fusion protein. In Western blots we detected a protein of M_r = 155 kd in chicken chondrocytes and of M_r = 140 - 150 kd in human and yeast. Long-term cultured cells produce a protein with lower molecular weight in comparison to the beginning of culture.
Immunofluorescence studies demonstrate a fine mesh-work resembling the one obtained by antibodies directed against Vimentin filament. Double staining experiments as well as different molecular weights showed that Catenin is codistributed with but not identical to Vimentin.

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Four calcium binding proteins in rat liver microsomes

Several important cellular functions are triggered by calcium release from internal stores. Recently it has been claimed the $InsP_3$-sensitive pool resicles in liver and pancreas acinar cells in a specific organelle ("calciosome") characterized by its content of the calcium binding protein calsequestrin[1].
Using immunological techniques we were unable to detect calsequestrin-like material in $InsP_3$-sensitive subfractions of rat liver microsomes. Instead we found four calcium binding glycoproteins with molecular masses of 59, 60, 80 and 90 kDa. The 59, 80 and 90 kDa proteins are of the high mannose type, the 60 kDa glycoprotein is of the mixed hybrid type with terminal galactoses .
All four proteins showed high affinity binding sites for calcium (K_D between 1 and 5 µM). The 80 and 90 kDa proteins possessed in addition 13 and 15 low affinity binding sites /molecule with K_D of 0.4 and 0.6 mM respectively.
On the basis of its calcium binding properties as well as its N-terminal sequence, the 60 kDa protein represents the rat liver equivalent to bovine calregulin[2].
The 90 kDa protein was identified as GRP94[3].
On the other hand, the 80 kDa and the 59 kDa proteins have no similarities with GRP78[3] and protein disulfide isomerase[3] respectively.

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The proteolytic processing of E. coli penicillin amidase

The E. coli penicillin amidase (PA; EC 3.5.1.11) is an important enzyme in Biotechnology with unknown physiological function. It is used industrially in the hydrolysis of penicillin G to 6-aminopenicillanic acid (6-APA), a precursor of the semi-synthetic β-lactam antibiotics. PA is a bacterial enzyme that is post-translational proteolytically processed. The proenzyme (87 k Dal) is cut into an α-chain (22 kDal) and a β-chain (65 kDal), by removing a piece of 53 amino acids from the primary chain.

The enzyme formed this way has an isoelectric point of 7.0 (= $PA_{7.0}$) and can be transformed autocatalytically to another active form (=$PA_{6.7}$; IP=6.7).

In E. coli homogenates and crude preparations of the enzyme at least five active protein-bands with different isoelectric points can be detected.

Poly- and monoclonal antibodies are used to examine

- the kinetics of the proteolytic processing in crude and isolated enzyme preparations
- the origin of all forms from a single gene product
- the enzyme pattern in E. coli homogenates of cultures in different growth phases and

- the immunological characteristics of chemically modified PA.

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Inositol 1,4,5-trisphosphate induces DNA synthesis in isolated plant nuclei

Inositolphosphates are active second messengers in animal cells. Although relevant work is on an early stage inositolphosphates seem to be part of a cellular regulation system in plants, too.

Isolated nuclei from barley seedlings incorporated ^{32}P-Orthophosphate and phosphate from ^{32}P-ATP in anorganic condensed pnosphates and inositolphosphates over a periou of nearly 3 hours. Among inorganic linear polyphosphates and cyclic metaphosphates, inositolphosphates of all phosphorylation grades (from mono- to hexakisphosphate) were detected. In a cell free system nuclei were incubated with synthetic Inositol 1,4,5-trisphosphate ($InsP_3$). Isolated nucleic acids were separated by acrylamide/agarose electrophoresis and monitored by UV scanning.

After a lag-phase of 15 min the amount of DNA increased and after 60 min it was twice the amount than in nuclei which had incubated without $InsP_3$ before. Since we compared in every experiment 2 identical nuclei suspensions, one incubated with $InsP_3$, the other one without, $InsP_3$ can be declared to be the agent which induces the doubling of DNA.

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Biogenesis of Soluble Glycoproteins of the tsO45 Mutant of Vesicular Stomatitis Virus at Permissive and Non-permissive Temperature

CHO cells when infected by the VSV tsO45 mutant of VSV at the restrictive temperature (39°C) produced two soluble glycoproteins (G_s and G_{ss}) both of which were found in the growth medium. In contrast, at the permissive temperature (31°C) only one soluble glycoprotein was formed. The soluble extracellular glycoproteins were endo H resistent and showed in SDS-PAGE higher mobilities compared to the mature authentic G protein of VSV tsO45. The differences of molecular weights between the authentic G protein and the soluble extracellular glycoprotein forms could be attributed to the loss of different segments at there carboxyterminal region using specific chemical cleavage by hydroxylamine at Asn-Gly linkages[1]. In pulse/chase experiments only one G_s protein was intracellularly detectable at 31°C and 39°C which remained endo H sensitive up to the end of the kinetic when its intracellular pool had considerable decreased. At 39°C a portion of the G protein formed trimers which were detected by sucrose gradient centrifugation and mature G protein appeared on the cell surface where it became accessible to polyspecific G protein antibodies. Particle preparations of the tsO45 mutant produced at 31°C contained one carboxyterminal fragment of the G protein which could be detected immunologically whereas particles harvested at 39°C contained at least two C-terminal fragments. Since there is no detectable intracellular pool of G_{ss} protein we speculate that this soluble G protein form is generated by proteolytic cleavage most probably at an extracellular site.

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Hypoxia Tolerance of Coronary Endothelial Cells

The response of endothelial energy metabolism to hypoxia was studied in coronary microvascular endothelial cells from rat. Suspensions of endothelial cells were incubated at defined Po_2 levels between 0.1 and 100 mm Hg by use of a special incubation technique, the oxystat [1].
In the presence of glucose (5 mM), endothelial respiration (4 nmol /min/mg cellular protein) was independent of the exterior Po_2 above 2 mm Hg; the oxygen consumption was half-maximal at 0.8 mm Hg. At a Po_2 of 100 mm Hg, the cells produced lactate at a rate of 25,8 nmol/min/mg cellular protein; decreasing the Po_2 to 0.1 mm Hg, resulted in an 2.2-fold increase. The contents of ATP, ADP, and AMP in nmol/mg cellular protein were 21.5 ± 2.6, 4.5 ± 0.7, and 2.3 ± 0.5, respectively; they remained constant for 2.5 h incubations at Po_2 levels between 0.1 and 100 mm Hg. In the presence of palmitate (100 µM) plus glutamine (0.5 mM), the rate of oxygen consumption was 8.1 nmol/min/mg cellular protein at Po_2 levels above 2 mm Hg, the half-maximal rate was again observed at 0.8 mm Hg. Lactate production, however, was negligible. At Po_2 levels above 2 mm Hg the energy state of palmitate plus glutamine supplied endothelial cells was the same as found in cells supplied with glucose only. At Po2 levels below 2 mm Hg, however, the adenine nucletide contents rapidly declined toward lower levels.
These results demonstrate that the respiration of coronary endothelial cells is not affected by hypoxia unless the exterior Po_2 drops below 2 mm Hg. Even at lower Po_2, the endothelial energetic state remains constant unless glucose is removed from the incubation medium.

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Characterization of a Guanine Nucleotide-specific Nucleoside Diphosphokinase in Bovine Rod Outer Segments

Light signal transduction in vertebrate rod outer segments (ROS) involves two GTP-hydrolyzing processes. First, the photoexcited receptor rhodopsin activates the G-protein transducin by promoting the exchange of the G-protein-bound GDP by GTP, which is subsequently hydrolyzed to GDP, which reaction terminates the active state of the G-protein. Second, GTP is used to form the intracellular signal cyclic GMP. Considering the relative concentrations of transducin and GTP in ROS, being both about 300 - 500 µM, and the large amplification of light signal transduction, it is feasible that activation of the vision cascade may lead to a rapid depletion of GTP. Since, however, total GTP concentrations in ROS are not markedly altered by even intense illumination, a nucleoside diphosphokinase (NDPK) (EC 2.7.4.6) has been postulated in ROS rapidly replenishing the used GTP.

We report here that bovine ROS membranes contain a highly active NDP phosphotransferase, which can be released from the membranes under conditions similar to those causing a release of transducin. The ROS enzyme exhibited the typical characteristics of a NDPK including the requirement of divalent cations and the formation of an abortive NDP-enzyme complex. The NDPK released from ROS membranes exhibited a 2-fold higher affinity for GDP than for ADP. The NTP's ATP and GTP gave similar maximal enzyme activities with a slightly higher affinity for GTP. The data suggest that the NDPK present in bovine ROS membranes is closely associated with transducin, the major GTP-hydrolyzing enzyme, and that the NDPK, by providing a constant level of GTP, may be an essential component of the whole light signal transduction system.

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Immunoaffinity Chromatography of Choline Acetyltransferase from Pig Brain

Choline acetyltransferase (ChAT) was purified from pig brain by conventional as well as by immunological means. Besides the well established methods for ChAT purification like CM-ionexchange chromatography or Blue Sepharose affinity chromatography we introduced a high pressure liquid chromatography based on hydrophobic interaction with matrix bound phenyl-groups. A polyclonal antiserum was raised against HPLC purified ChAT. By a non-dissociating, cathodic electrophoresis following HPLC purification we obtained highly purified ChAT in an analytical scale. In a preparative scale ChAT (350ug/10kg pig brain) was purified by a two step procedure taking one week: CM-ionexchange chromatography, immunoaffinity chromatography. For this method a highly specific monoclonal antibody was covalently linked to the sepharose matrix. The purification steps were monitored in addition to protein and enzyme activity measurement by Western Blot technique and immunodetection. By these procedures ChAT was shown to be contained in the starting material in extremely low amounts and thus does not accumulate substantially before higher purification factors are achieved.

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Specificity of a Cysteine Proteinase of Entamoeba histolytica against Various Unblocked Synthetic Peptides

Previous investigations have revealed that a cysteine proteinase of Entamoeba histolytica is able to cleave the Gly_{23}-Phe_{24} bond in the insulin B-chain (1) as well as one of the two glycine bonds present in the 1-CB2 peptide of the 1-chain of calf skin collagen (2), thus indicating that neighbouring amino acids are of importance for the susceptibility of peptide bonds on the carboxyl-terminal side of glycine. In the present study the activity of highly purified cysteine proteinase of E. histolytica against different peptides of the sequence X-Gly-Phe-Phe was compared. The synthetic peptide Arg_{22}-Gly_{23}-Phe_{24}-Phe_{25} of the insulin B-chain was readily hydrolyzed yielding Arg-Gly and Phe-Phe as split products. Lys-Gly-Phe-Phe and Tyr-Gly-Phe-Phe were cleaved at rates of 20 and 4%, respectively. Val-Gly-Phe-Phe, Gly-Gly-Phe-Phe, Glu-Gly-Phe-Phe and Ser-Gly-Phe-Phe were hydrolyzed at rates far below 1%. Gly-Arg-Phe-Phe, Gly-Phe-Phe and Gly-Phe were completely resistant to the proteinase. Another good substrate was found in Arg-Gly-Leu-Hyp, which

represents a model compound of a scissile site in collagen type I. Furthermore, peptide Arg-Arg-Phe-Phe was attacked by the enzyme releasing Arg-Arg and Phe-Phe. Compared with Arg-Gly-Phe-Phe at substrate concentrations of 2 mM the rates of hydrolysis of Arg-Arg-Phe-Phe and Arg-Gly-Leu-Hyp were 37 and 127%. The enzyme exhibited dipeptidyl peptidase activity against the nonapeptide Arg_{22}-Gly_{23}-Phe_{24}-Phe_{25}-Tyr_{26}-Thr_{27}-Pro_{28}-Lys_{29}-Ala_{30} of the insulin B-chain releasing Arg-Gly (3).

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Vectorial protein secretion in the polarized epithelial cell line MDCK

Epithelial cells display a characteristic polarization of their plasma membrane into two domains, the luminal membrane which in vivo faces the external milieu and the basolateral membrane by which the cell communicates with the internal milieu of the organism. The two plasma membrane domains exhibit different protein compositions, reflecting the capacity of these cells to vectorially transport newly synthesized proteins to either cell surface. To study the molecular mechanisms of this protein sorting process we analyse as a model system the vectorial secretion of proteins in the kidney derived cell line MDCK. We have identified and characterized an 80 kD glycoprotein complex (gp 80), which is secreted at the luminal side of the cell monolayer (1). Using this protein as a probe the roles of the carbohydrate moieties, the intravesicular pH and the cytosceleton in the luminal secretion was investigated (2-4). Furthermore, the cDNA coding for the gp 80 complex has been cloned. DNA sequence analysis revealed a striking homology to SGP 2, a protein secreted from the seminiferous testes epithelium. Both cDNAs shall be used for in vitro mutagenesis followed by the expression of the modified proteins in epithelial cells to identify those protein structures responsible for the specific targetting to the apical cell surface domain.

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The cytoplasmic domain is necessary and sufficient for targeting lysosomal acid phosphatase to lysosomes

Lysosomal acid phosphatase (LAP) is a glycoprotein predominantly located in lysosomes. A membrane associated and a soluble form can be distinguished. The membrane form (precursor) contains a large amino terminal domain, a membrane spanning domain and a short carboxyterminal cytoplasmic domain of 18 amino acids. LAP is synthesized and transported to lysosomes as a transmembrane protein. In the lysosomes it is converted into a soluble form by proteolytic cleavage [1,2]. Transport of LAP to lysosomes does not involve mannose 6-phosphate receptors [2]. The pathway of LAP to lysosomes includes the passage of the plasma membrane. Cell surface LAP is rapidly internalized. Most of the internalized LAP is transported back to the cell surface and undergoes several rounds of transport between the plasma membrane and the endosomes before it is transfered to dense lysosomes. At equilibrium 10-15% of the transmembrane form of LAP are present at the cell surface. LAP is transfered with an apparent half time of 6h from the plasmamembrane/endosome pool to dense lysosomes.
To investigate the molecular mechanism underlying the "alternative" way of transport to lysosomes C-terminal truncated mutants of LAP have been constructed. When the membrane spanning and cytoplasmic domains are truncated LAP

is secreted. When only the cytoplasmic domain is deleted the mutant LAP is retained intracellularly. More than 80% of this mutant LAP precursor accumulate at the cell surface and the time required for transport to lysosomes is more than doubled compared to wild type LAP. In a parallel study (B.Weber et al., this issue) it was shown that a chimeric molecule containing the luminal domain of the M_r 46000 mannose 6-phosphate receptor fused to the membrane spanning domain and cytoplasmic domain of LAP accumulates in dense lysosomes.
We conclude that the cytoplasmic domain of LAP is necessary for transport of LAP to lysosomes and sufficient to target a non-lysosomal protein to lysosomes.

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Preparation of a recombinant La-Protein from the marine sponge Geodia cydonium

The autoantigen La is known to be an abundant phosphoprotein associating transiently with the U-rich 3'-ends of a series of small nuclear and cytoplasmic RNAs forming ribonucleoprotein particles. During our studies on structure and function of La antigen we elucidated a monoclonal anti-La antibody (La11G5) which recognizes a 50-kD protein in human cells. With this antibody cross-reactive material was found in all eukaroytic species examined including mammals, rodents, amphibians, insects, protozoa and yeast, suggesting La antigen to carry epitopes highly conserved during evolution. Interestingly both the anti-La mab and a pool of patient anti-La sera also recognized the La antigen, a 43-kD protein, in total extracts of the marine sponge Geodia cydonium. To further characterize these data we constructed a cDNA library from this sponge using λgt11. We isolated two single plaque colonies of the putative phages by immunoscreening. After expression of the corresponding inserts in Y1089 lysogens and isolation of fusionprotein by immunoaffinity chromatography, we tested a collective of 41 sera of patients with autoimmune diseases containing anti-La antibodies. 32 sera were obtained from patients with SLE and 9 sera from patients with Sjögrens' syndrome. The sera were selected by screening for anti-La antibodies using counterimmunoelectrophoresis and immunoblotting. 38 sera of these sera recognized the recombinant La protein, supporting the hypothesis of conservation of the antigenic epitopes during evolution.

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F. Pilz und G. Auling

Induzierbarer Zinktransport bei dem ß-1,3-Glucan produzierenden Pilz Sclerotium rolfsii

Der filamentöse Pilz Sclerotium rolfsii kann extrazelluläre Polysaccharide zur Verwendung in der Lebensmittelindustrie oder im technischen Bereich nur bei ausreichender Versorgung mit Zink produzieren(1). Atomabsorptionsmessungen deuten darauf hin, daß der Pilz S. rolfsii Zink sehr stark auf der Myzeloberfläche akkumuliert. In Aufnahmeexperimenten mit dem Isotop $^{65}Zn^{2+}$ konnten wir demonstrieren, daß die intrazelluläre Akkumulation von Zn^{2+}-Kationen über ein energieabhängiges Transportsystem erfolgt. In Myzelien, denen bei der Anzucht mehr als 1 uM Zn^{2+} im Medium angeboten wurde, ist dieses Transportsystem nicht mehr nachweisbar. Gegenwärtig prüfen wir die Spezifität dieses induzierbaren Transportsystems. Der Zusammenhang zwischen Zinkakkumulation und ß-1,3-Glucanbildung wird in Zusammenarbeit mit der Arbeitsgruppe U. Rau / F. Wagner (Institut für Biochemie und Biotechnologie, TU Braunschweig) studiert.

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A. Pingoud, R. Geiger, J. Alves, T. Oelgeschläger and T. Rüter

Functional sites of the EcoRI restriction endonuclease probed by site directed mutagenesis

The EcoRI restriction endonuclease recognizes with high specificity the double stranded DNA sequence

$$\begin{array}{l} G^{\downarrow}AATTC \\ CTTAA_{\uparrow}G \end{array}$$

and in the presence of Mg^{2+} cleaves the DNA as indicated. A 3Å X-ray structure analysis of an EcoRI x DNA complex, crystallized in the absence of Mg^{2+}, indicates that the amino acid residues in positions 144 and 145 as well as 200 are involved in DNA binding [1]. Site directed mutagenesis experiments have shown that their involvement in the recognition process, however, is more intricate than proposed by the authors of the X-ray structure analysis [2-4]. In order to define more precisely the role of these and adjacent amino acids we have produced single and double mutants of EcoRI with amino acid replacements in position 144, 145, 147 as well as 199, 200, 203 and analyzed their activity in vivo [5] and in vitro after purification to homogeneity. The results of the in vivo assay allow to conclude that the structural integrity of the region at and around position 200 is very critical for the enzymatic function of EcoRI: non-conservative mutations lead to a dramatic decrease in activity. In contrast, the region around position 144 and 145 seems to be of minor importance for the enzymatic activity: non-conservative mutations in this region have similar moderate effects as conservative mutations. The analysis of the purified EcoRI mutants confirms these results. In addition, they demonstrate that some mutants which have a very low activity are still specific for the EcoRI site (Arg200 → Gly), while others show a relaxed specificity (Arg 200 → Glu or Gln and Asn199 → Asp). The Asn199 → Asp mutant, furthermore, attacks preferentially the central phosphodiester bond in its degenerate hexanucleotide recognition sequence. Taken together our results suggest that not only Glu144, Arg145 and Arg200 are involved in specificity determining interactions but other amino acids as well. The recognition process, furthermore, does not only depend on hydrogen bonds but also on the overall complementarity of charge dipoles, and presumably, is highly redundant.

We have begun to locate the catalytic center and the Mg^{2+} binding site of EcoRI. Two candidate regions were analyzed:

1. ^{92}G G I V E V K D - D Y G E W R
2. ^{126}L L V G K R G D Q D L M A A G

which show a homology to a consensus sequence proposed to be involved in Mg^{2+} binding in a variety of polymerases [6]. Preliminary results demonstrate that the second region is more critical than the first one for the catalytic action of EcoRI.

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B. Podschun and K.D. Schnackerz

Isotopic exchange reactions catalyzed by dihydropyrimidine dehydrogenase from pig liver

GABA (4-aminobutyric acid) is the major inhibitory neurotransmitter in the central nervous system. Evidence which is now accumulating indicates a similar role of ß-alanine (3-aminopropionic acid) (1). In mammalian tissues the only pathway leading to the biosynthesis of ß-alanine is the conversion of uracil via 5,6-dihydrouracil and N-carbamoyl-ß-alanine (2). In their predominant function the three pyrimidine catabolizing enzymes dihydropyrimidine dehydrogenase (EC 1.3.2.1), dihydropyrimidinase (EC 3.5.2.2) and ureidopropionase (EC 3.5.1.6) critically regulate the concentration of the free nucleic acid bases uracil and thymine (3). We have investigated the kinetic mechanism of dihydropyrimidine dehydrogenase purified from pig liver cytosol (4). Initial velocity studies in the absence and presence of products and dead-end inhibitors suggest a nonclassical ping-pong mechanism. The reaction consists of two half-reactions, namely the reduction of the enzyme by NADPH at the first site and the reduction of uracil at the second site connected by electron transfer via flavin and FeS clusters. Two separate sites are operative, one for each group of substrates and inhibitors. To support this mechanism we have measured the rates of isotopic exchange between NADPH and $NADP^+$ as well as between thymine and 5,6-dihydrothymine. ^{14}C-labeled $NADP^+$ was synthesized from [U-^{14}C]-NAD^+ using NAD^+-kinase and purified by DEAE-Sepharose chromatography. The NADPH/$NADP^+$-exchange follows a first order process and depends directly on enzyme concentration. The isotopic exchange between thymine and 5,6-dihydrothymine seems to be depressed at high dihydrothymine concentrations reflecting the stickiness of this reaction product.

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Purification and Characterization of the Glyceraldehyde-3-phosphate Dehydrogenase from Halobacterium vallismortis

Some extremely halophilic archaebacteria metabolize carbohydrates via a modified Entner-Doudoroff pathway[1]; the glycolytic pathway has not been demonstrated. A key enzyme of both reaction sequences is the glyceraldehyde-3-phosphate dehydrogenase. This enzyme has been purified from Halobacterium vallismortis. It occurs to be an enzyme, which catalyzes both, an NAD^+- and orthophosphate dependent oxidation of glyceraldehyde-3-phosphate (GAP) and an $NADP^+$-dependent, orthophosphate independent oxidation of GAP. The relation of both activities is almost constant during purification.

The three step purification includes chromatographies on Sepharose Cl-6B, hydroxylapatite and octylsepharose. The enrichment was more than 100 fold with a recovery of 20 - 30 %. Polyacrylamidegelelectrophoresis exhibits nearly homogenity of the protein.

The molecular weight of the native enzyme is about 140 kDa as determined by gel filtration; SDS-PAGE gives a value of 70 kDa, cetyltrimethylammoniumbromide-PAGE leads to a somewhat lower value. Both results are in accordance with a dimeric structure of the native protein.

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Potassium Transport in Escherichia coli: Site-directed Mutagenesis of the KdpB Subunit of the Kdp-ATPase

The Kdp-ATPase of Escherichia coli is a high-affinity K^+-uptake system (K_m= 2 µM), the expression of which is turgor regulated. The kdp genes are located at 16 minutes on the E.coli chromosome and organized as a regulon of 8kb size. This regulon consists of the kdpABC operon encoding the structural proteins KdpA (59 169 Da), KdpB (72 112 Da), KdpC (20 267 Da) and the kdpDE operon coding for the regulatory proteins[1]. The kdpABC genes have been cloned and sequenced[2].

The DNA sequence of the kdpB gene disclosed homologies to other eukaryotic P-type ATPases like the Ca^{2+}-ATPase of sarcoplasmic reticulum, or the H^+-ATPase of yeast plasma membrane.

The phosphorylation site of the Kdp-ATPase is located in the KdpB subunit[3]. Based on homology comparisons the most probable candidate for phosphorylation is Asp 307, being located in a highly conserved region of the polypeptide chain.

Applying the method of site-directed mutagenesis, based on the method of Eckstein and coworkers, we generated substitutions of the Asp 307 residue to Glu, Gln, Ala, His, Asn, Tyr, Ser, Leu, Val. The mutated kdpB genes were subcloned from M13 phage (in which mutagenesis and sequencing was done) into a pBR322 derivative. The effect of those plasmid coded mutations on growth behavior, potassium uptake, expression, ATPase activity and phosphorylation capacity of the KdpB subunit have been studied.

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Quantitative Isomer 'Profiling' of Inositol Phosphate Metabolites in Avian Erythrocytes. A Study Combining NMR Spectroscopy and HPLC with Direct Isomer Detection

Inositol phosphate isomers were isolated in micromol quantities from turkey blood by a large scale anion exchange chromatography and subjected to proton NMR and HPLC analysis. We employed a HPLC technique with a novel detection system, called 'metal-dye detection'[1], which is useful to identify and quantify non-radioactively labeled inositol phosphate isomers. Although enantiomeric structures where not determined, the results indicate that avian erythrocytes contain the same inositol phosphate isomers as mammalian cells, i.e. Ins(1,4)P_2, Ins(1,6)P_2, Ins(1,3,4)P_3, Ins(1,4,5)P_3, Ins(1,3,4,5)P_4, Ins(1,3,4,6)P_4, Ins(1,4,5,6)P_4, Ins(1,3,4,5,6)P_5, and InsP_6. Except Ins(1,3,4,5,6)P_5, which occurs in millimolar amounts in avian blood, most other isomers are present only in a micromolar concentration. Two more identified inositol trisphosphate isomers were hitherto not described for mammalian cells: Ins(1,5,6)P_3 and Ins(2,4,5)P_3. The position of these two isomers in inositol phosphate metabolism is unknown.

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J. Radons and A. Hasilik

Separation of functionally different 215-kDa mannose 6-phosphate receptor containing compartments from U 937 promonocytes

The segregation of the biosynthetic precursors of soluble lysosomal enzymes from secretory products depends on the formation of mannose 6-phosphate residues in the N-linked carbohydrate side chains in the precursors and their binding to specific mannose 6-phosphate receptors. In most cells examined so far, two receptors have been found: a cation-independent 215-kDa ("large") receptor, which is involved in intracellular sorting and in endocytosis, and a cation-dependent 46-kDa receptor, which probably participates in the intracellular sorting. The large receptor has at least two binding sites. Besides mannose 6-phosphate containing ligands it binds and internalizes insulin-like growth factor II. In acidic compartments such as CURL or endosomes the ligands dissociate from the large receptor and are delivered to lysosomes while the receptor is recycled to the Golgi apparatus or to the plasma membrane. It has been shown previously that the large receptor is exchanged between the involved compartments; at 37 °C essentially all receptors have an access to antibody present in the medium. In order to characterize biochemically the compartments exchanging the large receptor, we developed a metrizamide-containing gradient, in which a characteristic distribution of β-hexosaminidase, galactosyltransferase and the receptor was obtained for fractions from U 937 promonocytes. In this gradient we studied the distribution of the large receptor following the binding or binding and internalization of a ^{125}I-labelled anti-receptor monoclonal antibody (2C2). Depending on temperature and duration of the incubation with the antibody the latter was recovered in different gradient fractions. When the incubation was performed at 18 °C the labelled receptor was found only in a rapidly sedimenting compartment, while at ≥ 22 °C the label became progressively associated with a slowly sedimenting compartment. We conclude that our gradient centrifugation method is suitable for the separation of functionally different 215-kDa mannose 6-phosphate receptor containing compartments.

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Bodo Rak and Karin Schnetz

The β-glucoside-specific permease - an integral membrane protein - negatively controls expression of the β-glucoside (*bgl*) operon of E.coli

The *bgl* operon encodes all specific functions necessary for regulated uptake and degradation of certain aryl-β-glucosides. We have determined the nucleotide sequence of the operon and functionally defined the order of its genes[1]. The first gene, *bglG*, encodes a positive regulatory protein, the second one, *bglF*, the transport protein (enzyme II^{bgl} of the PTS) and the third, *bglB*, a phospho-β-glucoside hydrolase. The operon is preceded by two promoters, the first of which is cryptic in the wildtype but can be activated by an enhancer-analogous mechanism[2]. This promoter reads the operon in the induced state. Substrate-dependent induction of the operon has no major effect on the initiation of transcription but acts via transcriptional antitermination at two terminators which bracked gene *bglG*. The product of *bglG* is responsible for specific antitermination. A second, enhancer-independent promoter, which is located within the first terminator, may serve to ensure low-level synthesis of *bglG* and *bglF* product to keep up inducibility. Deletions of gene *bglF* turned out to be constitutive in antitermination revealing the additional role of the transport protein as a negative regulator of the operon. Results from site-specific mutagenesis and complementation studies suggest the following scenarios:
(i) in presence of substrate protein HPr phosphorylates histidine residue 547 of enzyme II^{bgl}. This phosphate group is immediately transferred to histidine residue 306 and from there to the transported sugar. Thus, residue 547 is only transiently phosphorylated.
(ii) in absence of substrate the phosphate group present at residue 547 cannot be drained by sugar phosphorylation and is now being used to inactivate antiterminator activity of *bglG* gene product, probably by phospho-transfer to protein BglG.

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G.Rapp, J.Klaudiny and K.H.Scheit

Characterisation by cDNA cloning of a highly abundant mRNA from human ovarian granulosa cells

A number of cDNA clones from a human ovarian granulosa cell cDNA library [1,2] were subjected to Northern analysis. cDNA clone pHGR74 contained an insert of approx. 900bp which identified a highly abundant mRNA species of the size 920bp in poly(A$^+$)RNA from human granulosa cells. This indicated that cDNA clone pHGR74 represented a full length cDNA of the respective mRNA species. The sequence of the insert of cDNA clone pHGR74 possesses a short poly(A) tail with a polyadenylation signal AATAAA 14 nucleotides upstream of the poly(A) tail. Computer analysis identified two open reading frames (ORF) each starting with a methionine. The coding regions extends for ORF(A) from nucleotide residues 318-648, for ORF(B) from residues 367-435. For ORF(A) a protein sequence of 111, for ORF(B) a sequence of 57 amino acid residues was deduced. In favour of the reading frame (A) is the fact that the start codon ATG is part of the sequence CATCATGG which has the characteristic features of a consensus sequence apparently controlling the translational efficiency of mammalian mRNAs (3,4,5). Neither DNA sequence of pHGR74 nor those of the deduced amino acid sequences of ORF(A) and ORF(B) have yet been reported in sequence data banks.
Northern blots of poly(A$^+$)RNA from porcine corpus luteum, human granulosa cells, human prostate and human testes when probed with the cDNA insert of pHGR74 yielded strong signals in human granulosa cells as well as in human testes only. This preliminary result indicates both a likely tissue and species specificity for the distribution of the mRNA specified by cDNA clone pHGR74

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Rafael Ratajczak and Wolfgang Haehnel

Control of Photosynthetic Electron Transport at Low pH Values by Inactivation of Plastocyanin?

The photosynthetic electron transport rate is decreased at low pH values in the thylakoid lumen. The site of this control is at or near the cytochrome b_6/f-complex (1) by a mechanism which is not fully understood. Plastocyanin functions as the electron acceptor of the cytochrome b_6/f-complex. Its reduced form is protonated at His 87 with a pK of 5.4 which leads to a trigonal structure of the Cu-center (2) and an inhibition of its oxidation. We have studied if this conformational change may control the electron transport rate at low pH values as proposed (2). We have measured the electron transfer from plastocyanin to PS I in inside-out vesicles and in isolated stroma lamellae in the presence of the low detergent concentration of 0,05 % Triton X-100 (3) as a function of pH. The second order rate constant shows at pH 4.5 and 5 maximum values of $1.8 \cdot 10^8$ $Mol^{-1}s^{-1}$ and 10^9 $Mol^{-1}s^{-1}$, respectively. This effect is consistent with a diminished electrostatic repulsion of the proteins at low pH but not with an inactivation of reduced plastocyanin. The electron transfer from chemical cross-linked plastocyanin to PS I with a halftime of 14-17 μs is not pH dependent. We conclude that inactive reduced plastocyanin at low pH values is immediately converted to the active form if plastocyanin interacts with PS I and that His 87 of reduced plastocyanin is not protonated in the reaction complex at physiological pH values.

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2,8-Diazido ATP - a cross-linking photoaffinity label for ATP synthase complexes

Strong catalytic site cooperativity could be demonstrated for the synthesis/hydrolysis of ATP catalyzed by various ATP synthases (EC 3.6. 1.34). The catalytic part (F_1ATPase:$\alpha_3\beta_3\gamma\delta\varepsilon$) of these enzyme complexes usually has six nucleotide binding sites (three catalytic and three noncatalytic) on the major subunits α and/or ß. For an effective cooperativity between these nucleotide binding sites subunit subunit interactions are indispensable. Strong subunit subunit interactions should be guaranteed best by the localization of the nucleotide binding sites directly at the interfaces between two subunits. Thus all catalytic or regulatoric events at interfacial sites immediately influence the adjacent subunits.

First experimental evidence for an interfacial localization of nucleotide binding sites between α- and ß-subunits has been obtained by photoaffinity cross-linking of various ATP synthases with bifunctional photoactivatable ATP analogs. Upon irradiation of the enzymes in presence of 2,3'-DiN_3ATP or 8,3'-DiN_3ATP a nucleotide specific formation of α-ß cross-links was observed [1-3]. Nevertheless, this proof for an interfacial localization of nucleotide binding sites is not unambiguous due to a maximal distance of ~2 nm between both azido groups.

For this reason we have synthesized 2,8-diazido ATP (2,8-DiN_3ATP) with both azido groups directly at the adenine ring. 2,8-DiN_3ATP is hydrolyzed by F_1ATPase from the thermophilic bacterium PS3. The irradiation of this enzyme in the presence of 2,8-DiN_3ATP results in the nucleotide specific inactivation and in the nucleotide specific formation of higher molecular weight cross-links, obviously composed by two of the major subunits α and ß. This cross-link formation is the first clear proof for the localization of nucleotide binding sites directly at the interface between α- and ß-subunits.

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Michael K. Renner, Hans-Jürgen Hinz, Dieter Hansen and Wolfgang Hillen

Specific Tetracycline Binding to Tet Repressor can Eliminate Stability Defects Introduced by Point Mutations

Tet repressor (1,2) stability was determined by high sensitivity differential scanning microcalorimetry(3,4) for wild type repressor

and three mutants having Trp 75 to Phe 75, Trp 43 to Phe 45, and Trp 75, Trp 43 to Phe 75, Phe 43 exchanges, respectively. Studies were performed in 50 mM cacodylate buffer pH=8.5 in the absence and presence of tetracycline. Stability was characterized by transition temperatures, transition enthalpies and transition entropies derived from the heat capacity vs. temperature curves.

In the absence of the tetracycline Phe 43 mutation does practically not affect the transition temperature or the transition enthalpy, whereas the Trp 75 to Phe 75 exchange reduces the transition temperature by 7.7 degrees and the transition enthalpy by 22 kJ/(mol of monomer) relative to the parameters of the wild type. The repressor having both point mutations shows stability properties almost identical to those of the Trp 75 to Phe 75 mutant.

In the presence of an excess of tetracycline the transition temperatures of wild type and all mutants are raised between 17 to 23 degrees indicating binding of the inducer to the repressor proteins. The association constants for formation of the various complexes between tetracycline and the four Tet repressors at the corresponding transition temperatures have been calculated from the transition parameters.

Relative Gibbs free energies of stabilization were estimated from the calorimetrically determined transition entropies and the shifts in transition temperature of the proteins resulting from mutations or complexation by tetracycline.

Proper analysis of these parameters for the various mutant proteins in the presence and absence of the ligand demonstrated the ability of tetracycline to overcome stability defects resulting from the point mutations.

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Stability Studies on BPTI Analogs

Dynamics and stability of proteins are largely determined by non-covalent interactions such as hydrophobic forces, hydrogen bonds, salt bridges, van der Waal's attraction and repulsion, hydration and dipol-dipol interaction. The only covalent interation of biological significance are disulfide bridges. A characteristic property of proteins is their marginal stability that renders them susceptible to small changes such as point mutations. Design of recombinant proteins having novel functions, higher stability or increased activity requires the understanding of the energetic, structural and functional effects of amino acid exchanges.

In the course of our studies on bovine pancreatic trypsin inhibor[1,2] we have shown that removal of the disulfide bridge between Cys 14 and Cys 38 by reduction and subsequent carboxymethylation (BPTI-RCOM) or carboxamidomethylation (BPTI-RCAM) results in a drastic decrease of the Gibbs free energy of stabilization by approximately 70% at pH = 2. The decrease in DG originates mainly from significant decreases of the stabilizing interactions which are reflected in the transition enthalpies DH. This finding is at variance with the generally held belief that disulfide bridges stabilize preferentially entropically via reduction the number of degrees of freedom of the unfolded state.

The previous experiments were subject to criticism since the BPTI analogs contained bulkier groups than the native inhibitor, which by their presence could already perturb the structure of the protein. Therefore we started to study BPTI-Ala 14,38, which contains Ala residues instead of the Cysteines in positions 14 and 38. Preliminary differential scanning calorimetry studies demonstrate that BPTI-Ala 14, 38 is apparently even less stable than BPTI-RCAM and BPTI-RCOM under identical solution conditions. This result is in accordance with recent findings on barnase (3,4) which also provided evidence for the fact that replacement of larger residues by smaller ones reduces favourable interactions, thereby decreasing overall stability.

The influence on stability of covalent interactions in BPTI was further investigated by studying BPTI*, a BPTI analogue that has the bond between Lys 15 and Ala 16 cleaved but all 3 disulfide bonds intact. The stability of BPTI* is significantly reduced to approximately 10% of that of the native molecule.

As mentioned above non-covalent interactions are of major importance for the structural and functional integrity of proteins. We investigated another BPTI analogue in which the 4 surface lysines had been changed into homoarginines. This is a particularly interesting alteration, since thermophilic behaviour of some enzymes has been discussed as being possibly dependent on their Arg to Lys ratio at the surface of the proteins. Our studies on this BPTI analogue did, however, not detect any stability increase resulting from the replacement. This may be a consequence of the fact that native BPTI appears to realize optimal stability and that therefore any change will result in a decrease in the number of favourable interactions.

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P. Richard and P. Gräber

Reconstitution of the ATP-Synthase from Chloroplasts

The ATP-synthase from 'chloroplasts has been isolated and reconstituted earlier into asolec-

tin liposomes by detergent dialysis. These proteoliposomes show high ATP-synthesis activity. However, in the resulting proteoliposomes the distribution of the protein on the liposomes is inhomogeneous and after a $\Delta pH/\Delta\Psi$ jump the transmembrane energization declines within about 300 ms. Therefore, we have investigated different lipids and reconstitution procedures. A mixture of phosphatidylcholin phosphatidic acid has been used as lipid. Then, liposomes have been prepared by detergent dialysis and a homogeneous vesicle distribution was obtained by repetitive filtration. Finally, CF_oF_1 and detergent was added. Removal of the detergent leads then to homogeneous liposome preparation. After energization by a $\Delta pH/\Delta\Psi$ jump, a constant rate of ATP synthesis is observed up to 2 s. The maximum rate obtained up to now is about 60 ATP/(CF_oF_1 s).

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Photoaffinity cross-linking of ATP synthase by 5'-p-fluorosulfonylbenzoyl-8-azidoadenosine

An interfacial localization of the nucleotide binding sites of ATP synthases [F_1ATPases (EC 3.6.1.34)] between α- and/or ß-subunits yields an attractive model to explain the strong catalytic cooperativity of the nucleotide binding sites during ATP synthesis/ATP hydrolysis. The experimental evidence for an interfacial localization obtained by photoaffinity cross-linking of various ATP synthase complexes with 3'-arylazido-2- or 8-azido ATP (2,3'-diN_3ATP and 8,3'-diN_3ATP) is vague due to a maximal distance of about 2 nm between both azido groups [1-3].

Two strategies for proofing an interfacial arrangement of nucleotide binding sites can be followed: Fristly, by a diminution of the maximal distance of both reactive groups as realized by the synthesis of 2,8-diN_3ATP [4]; secondly, by the incorporation of two functional groups into the affinity label which are expected to react directly at the nucleotide binding site. The esterification of p-fluorosulfonylbenzoic acid with 8-azidoadenosine results in the formation of the bifunctional 5'-p-fluorosulfonylbenzoyl-8-azidoadenosine (FSB-8-N_3A). The photoreactive 8-azido group should react directly at the binding site for the adenine ring, whereas the reactive fluorosulfonyl group should label the enzyme at the binding site for the γ-phosphate of ATP [5].

Incubation of F_1ATPase from Micrococcus luteus in the dark with FSB-8-N_3A results in the reduction of enzymic activity due to the reactive fluorosulfonyl group of the label. Additional irradiation leads to increased inactivation of the enzyme and to the formation of higher molecular weight cross-links (α-ß, ß-ß). Addition of ATP or ADP protects the enzyme against the attack of the label.

Supporting the results obtained with 2,8-diN_3ATP [4] the nucleotide specific formation of α-ß and ß-ß cross-links by FSB-8-N_3A demonstrates the interfacial localization of nucleotide binding sites of F_1ATPases directly between the major subunits α and/or ß.

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Metabolically separated succinate pools in muscle tissue

When isolated rat diaphragms were incubated with [1-^{14}C]-propionate, incorporation of radioactive label into malate and subsequent citric acid cycle intermediate pools proceeded at a much faster rate than into the succinate pool (1). In order to investigate the underlying mechanisms, we compared the different fates of label from the C-1 and C-2 position of propionate and studied the specific radioactivity of acyl-CoA intermediates.

Diaphragms were incubated with 50µM 1- or 2-^{14}C-labelled propionate (0-60 min). Label incorporation into metabolites was then examined as detailed previously (2). Acyl-CoA intermediates were separated by reversed phase high performance liquid chromatography (3).

With [1-^{14}C]- and [2-^{14}C]propionate, a similar time course of label incorporation was found. With [2-^{14}C]propionate, release of $^{14}CO_2$ was about 60% lower, but the steady state specific radioactivities of tissue metabolites were distinctly higher (50-200%). Label incorporation into acyl-CoA intermediates was determined after 15 min of incubation.

With both 1- and 2-^{14}C-labelled propionate, the specific radioactivity of propionyl-CoA was much higher than that of all other ^{14}C-labelled compounds (5-10-fold). However, the specific radioactivity of succinyl-CoA was found to be about 5-fold higher than that of succinate and only twice that of malate. Label from [2-^{14}C]propionate was additionally recovered in acetyl-CoA.
The results indicate that two separate succinate pools of different magnitude exist in muscle tissue. A smaller metabolic active pool shares the citric acid cycle. The significance of the much larger, presumably extramitochondrial pool is unclear.

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C.Sabelhaus and M.Mäder

Purification and Immunoblotting of Choline Acetyltransferase from Human Brain

Choline acetyltransferase (ChAT) was purified by conventional means. By CM-Sephadex ionexchange chromatography, Blue Sepharose affinity chromatography and high pressure liquid chromatography with TSK-Phenyl a purification factor of 2000 was achieved. After SDS-PAGE of the purified ChAT-samples the proteins were transferred onto nitrocellulose (Western blot). With a monoclonal antibody raised against pig brain ChAT a single band with 67-68 kD was detected. However, the Coomassie-stained protein pattern on the gel showed that human brain ChAT has not yet been purified to homogeneity.
By comparison of human ChAT to pig ChAT with respect to either equal protein or equal activity concentrations by immundetection with the above monoclonal antibody on Western blot the high specificity of this antibody towards the pig brain antigen and the very low cross-reactivity with the human antigen was shown. These results were additionally confirmed by the ELISA-method. Over 100-fold the amount of human ChAT activity is necessary to reach similar adsorbance rates as with pig brain ChAT.
These results confirm that for further purification and characterisation of human ChAT particularly by immunological means the development of specific monoclonal antibodies is essential.

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Protein Import in Chloroplasts: An Outer Envelope Protein is Synthezised and Translocated in the Absence of ATP and without Cleavable Transit Sequence

We have isolated a full length cDNA clone from a lambda gt 11 library using an antiserum raised against total chloroplast envelope protein. The in vitro translation product of this clone gives a protein of about 6.5 kDa. Import studies show that this protein integrates into the outer chloroplast envelope membrane, also in the absence of ATP. Processing of the protein during or before integration into the membrane was not observed, thus indicating that the protein does not have a cleavable transit sequence. Pretreatment of intact chloroplasts with protease (thermolysin) does not abolish protein translocation as in control experiment using stromal precursor proteins, suggesting a thermolysin resistant import pathway. Our results indicate also that this cDNA corresponds to the "10 kDa" outer envelope membrane protein of spinach described by Joyard et al. ((1982) J.Biol.Chem. 257,1095-1101). The protein shows abnormal migration properties in SDS-PAGE. Depending on the amount of protein loaded onto the gel it migrates between 6.5 and 14 kDa.

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Channel Structure of Porin OmpF as Visualized by High-Resolution Cryo-Electron Microscopy

Porin OmpF is an integral membrane protein from the outer membrane of Escherichia coli which forms 2D lattices in reconstituted vesicles. Electron microscopy studies on negatively stained specimens clearly show three channels traversing the membrane. No information about the protein, known to consist to 65% of ß-pleated

sheet structure, could be revealed by these investigations. To visualise the projection of the porin trimer we used glucose embedded 2D lattices, improved by phospholipase A2 treatment. The imaging and the electron diffraction studies were performed with the Siemens cryomicroscope, equipped with a superconducting objective lens. The electron diffraction pattern shows a resolution of 0.32 nm and the best image up to 0.35 nm. By combining amplitudes from el. diffraction data and phases from images a density map of the porin trimer at a resolution of 0.5 nm has been calculated. This map clearly shows a ß-pleated sheet delineating the protein-lipid interface as a density band 0.6 nm thick on average. Another intriguing feature is the low density area in the center of the trimer.

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Kinetic characterization of human branched-chain amino acid aminotransferase activity

The transamination of L-leucine, L-valine, L-isoleucine, and L-allo-isoleucine (BCAA) and their derived 2-oxo acids (BCOA) is catalysed by branched-chain amino acid aminotransferase (EC 2.6.1.42). Inherited deficiencies in activity are documented. However, reliable data on the kinetic properties are not available (1). We here report on the kinetic constants of the human skin fibroblast enzyme.

Enzyme activity was solubilized from cultured normal cells. Velocity studies were carried out in incubations with [1-^{14}C]BCAA (0.25-5.0 mM) or BCOA (0.05-1.0 mM) as substrates (5 concn., pH 8.3, 37° C). 2-Oxoglutarate (0.5-20 mM) and L- glutamate (5-100 mM) served as amino group acceptor and donor (5 concn.), respectively. Reaction rates were determined by measuring [1-^{14}C]BCOA and BCAA production, respectively (2). Appropriate blanks were run in parallel.

Each set of data was consistent with a Ping Pong Bi Bi mechanism and was therefore evaluated essentially as given by Henson & Cleland (3). For the amino acids, the following apparent K_m values were found (data for the respective 2-oxo acids are given in parentheses): L-leu, 0.6 mM (0.5 mM); L-val, 3.2 mM (0.4 mM); L-ile, 0.7 mM (0.3 mM); L-allo-ile, 1.8 mM (0.3 mM). Values for L-glu depended on the BCOA present and amounted to 30 mM (4-methyl- and (S)-3-methly-2-oxopentanoate) and to about 10 mM (3-methyl-2- oxobutanoate, (R)-3-methyl-2-oxopentanoate). Apparent Km values for 2-oxoglutarate were in the range of 3-5 mM. Apparent V_{max} (in nmol/min per mg of cell protein; data for expts. with the derived 2-oxo acids in parentheses) in experiments with L- leucine, L-valine, L-isoleucine, and L-allo- isoleucine amounted to 10 (15), 13 (5), 17 (10), and 8 (3), respectively.

Taken together, the data indicate that formation of amino acids is favoured not only thermodynamically but also kinetically over 2-oxo acid production under physiological conditions.

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Structure of the complexes of *E. coli* single-stranded DNA binding protein (*E. coli* SSB) with polymeric nucleic acids as observed in hydrodynamic studies

E. coli SSB protein plays a vital role in replication, recombination and repair processes. It binds strongly and co-operatively to all kinds of single-stranded nucleic acids. For the homopolymer poly(dT) and for native single-stranded DNA as e.g. phage DNA the affinity is of the order of 10^{10-11} M^{-1}. This strong binding somehow contradicts the neccessary dynamics of single-stranded nucleic acis in the physiological reactions.

We investigated the structure of the complexes of *E. coli* SSB with synthetic poly(dT) and native, circular single-stranded DNA of the filamentous phage M13mp8 by static and dynamic light scattering as well as by analytical ultracentrifugation.

For poly(dT) we could show , that binding of *E. coli* SSB to the polymer does not change the hydrodynamic radius of the polymer significantly. The free DNA forms a random coil which at low ionic strengths is swollen due to the repulsion of the negative charges of the phosphate backbone. At higher ionic strengths the structure is more compact. Independently of this effect binding of *E. coli* SSB simply replaces water trapped within the polymer. Unfortunately the poly(dT) available was too short (1400 bases) to permit dynamic light scattering to detect any internal movements.

The structure of M13mp8 single-stranded DNA (7229 base pairs) is somewhat more complicated. 40 - 70 % of the bases are engaged in base-paired secondary structure. The stability of these structures strongly depends upon the ionic strength and thus influences the apparent binding site size of the *E. coli* SSB. The overall shape of the *E. coli* SSB saturated DNA is not very different from that of the ssDNA alone but with higher ionic strengths fewer bases are available for *E. coli* SSB binding. The size of the phage DNA is of the order of the reciprocal length of the scattering vector and thus internal movements can be observed by dynamic light scattering. Binding of saturating amounts of *E. coli* SSB to M13mp8 DNA does reduce the amplitude of internal movements of the DNA but does not change the time scale of these movements. We conclude, that in solution the complex of native

ssDNA with *E. coli* SSB does resemble a string of pearls but that between these pearls the DNA retains its original high flexibility.

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Bettina Scheel and Klaus Kloppstech

Diurnal oscillations in the levels of light-inducible mRNAs in the polysomes of pea

In a previous publication we have shown that mRNA levels for light-inducible proteins oscillate in a diurnal and circadian fashion[1]. The question, however, could not be answered whether correspondent fluctuations would also occur at the level of translated proteins in vivo. Provided that mRNAs initiated into ribosomes are actively translated we isolated polysomes from light-dark grown pea at 4 hour intervalls during the day. Polysomes were isolated using two different methods of centrifugation in order to isolate the heavy or the light polysomal fractions, respectively. The RNA was isolated by digestion with proteinase K in SDS and phenol extraction; selection of poly(A)RNA was avoided in order to obtain the total initiated mRNA fraction. This RNA was dotted onto nylon membranes and hybridized against labeled inserts of the following clones: LHC II, SSU, ELIP and Actin[2], as a reference for a non-oscillating house-keeping protein. We could show that actin does not fluctuate during the day. In contrast to that all of the light-inducible proteins oscillate in a way very similar to that observed for poly(A)RNA. This clearly indicates that the translation of these proteins follows the amounts of mRNA available for each individual protein during the day.

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Synthesis and radical degradation of hyaluronate by synovia from patiens with rheumatoid arthritis

The viscosity of synovial fluid of patients with rheumatoid arthritis is reduced due to a reduced molecular weight distribution of hyaluronate (1,2).
We have shown that hyaluronate is degraded by superoxide, generated from macrophages. There was no indication that hyaluronate was degraded in the synovial fluid (3).
We have devised a procedure which could distinguish between dissociation of complete hyaluronate-chains and degradation: Growing hyaluronate-chains were pulse labeled in organ cultures and the molecular weights were determined during the pulse period.
The elution profiles led us to conclude that hyaluronate was degraded during synthesis. Large hyaluronate-chains were synthesized initially and released as fragments from the synovial membranes. These results were compared with synovial membranes obtained from patiens with arthrosis. These synovia produced less hyaluronate which was of higher molecular weight.

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Impaired Proteolytic Processing of Cathepsin B in Fibroblasts from Patients with Sialic Acid Storage Disease

In sialic acid storage disease (SASD), a defective transport system in the lysosomal membrane results in intralysosomal accumulation of free sialic acid(1). An impaired proteolytic processing of two lysosomal enzymes was found in fibroblasts from patients with SASD, the mature enzymes being about 2 kDa larger than those from normal cells(2).

We are studying the processing steps and molecular forms of cathepsin B in fibroblasts from patients with SASD and compare the results with those obtained with normal fibroblasts. Metabolic labelling of fibroblasts followed by immune precipitation, SDS-PAGE and fluorography showed that in SASD fibroblasts the single-chain mature form of cathepsin B has an M_r of 34 kDa, the two-chain form 28 kDa, compared with 33 and 27 kDa, respectively, in normal fibroblasts(3). Treatment with peptide:N-glycosidase F did not eliminate the molecular weight differences, suggesting impaired proteolytic maturation. Enzyme activity of cathepsin B in SASD fibroblasts was within the normal range. To investigate whether the differences in proteolytic processing are caused by secondary effects of the storage material or by altered properties of procathepsin B, cross-endocytosis experiments were performed using NH_4Cl-induced, [^{35}S]methio-

nine-labelled secretions from SASD and control fibroblasts. Both normal and SASD cells as recipients processed the endocytosed procathepsin B to their respective mature forms.

In conclusion, the abnormal mature forms of cathepsin B in SASD fibroblasts are probably due to a defect in the equipment for proteolytic processing. We are presently investigating, whether this defect is limited to the lysosomal compartment or is present already in an earlier compartment on the way to the lysosomes.

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K.Schmid und J.W.Lengeler

Konstruktion eines Hybrid-Operons für den Stoffwechsel von Sucrose und Raffinose in Enterobakterien

Metabolische Plasmide können das Substratspektrum ihrer Wirtszellen erweitern. So ermöglicht das Raf-Plasmid pRSD2[1] *E. coli* K-12-Zellen Wachstum auf Raffinose (nicht jedoch auf Sucrose), das Scr-Plasmid pUR400[2] Wachstum auf Sucrose (nicht jedoch auf Raffinose). Durch Insertion *raf*-spezifischer Strukturgene von pRSD2 in das *scr*-operon von pUR400 wurde *in vitro* ein *scr*/*raf*-Operon konstruiert, das *E. coli*-Zellen den Abbau von Raffinose und Sucrose ermöglicht. Alle Strukturgene in diesem Operon sind sowohl durch Raffinose als auch durch Sucrose induzierbar, die *raf*-Gene jedoch geringer als die *scr*-Gene. Diese Diskrepanz könnte darauf beruhen, daß die Translationseffizienz der *raf*-Gene (noch) nicht an die Transkriptionsrate des *scr*-Operons angepaßt ist.
Nach stabiler Integration des *scr*/*raf*-Operons ins Chromosom von *E. coli* soll beobachtet werden, wie und wie schnell eine Optimierung der Genexpression dieses Hybrid-Operons *in vivo* erreicht wird.

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Receptor mediated uptake and antiproliferative potency of arterial smooth muscle cell heparan sulfate

Proteoheparan sulfate isolated from the cell layer of cultured arterial smooth muscle cells has been shown to be a cell membrane integated molecule, the ektodomaine of which bearing 3-4 heparan sulfate side chains[1]. In the course of the turnover 4o% of the cell associated proteoheparan sulfate are released into the culture medium leaving a significant part of its protein core in the cell membrane. The extracellular proteoheparan sulfate is internalized by its parent cells via a receptor mediated process. The receptor which recognizes the heparan sulfate side chain but not the protein core crossreacts with an antibody generated against a heparin/heparan sulfate binding glycoprotein isolated from bovine uterus.

Interaction with the smooth muscle cell receptor of proteoheparan sulfate or its heparan sulfate side chains causes a 50% inhibition of cell proliferation when present in a concentration of 5 microgram / ml medium and is thus 40 fold more effective than heparin. The finding that heparan sulfate in this concentration has - in contrast to heparin - no anticoagulant activity leads to the conclusion that there are different structural requirements for antiproliferative and anticoagulant potency of heparan sulfate and heparin, respectively.

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The Shikimate Pathway is Active in Non-Photosynthetic Tissue of Plants

The activities of 5 shikimate pathway enzymes, dehydroquinate synthase (EC 4.6.1.3), dehydroquinate hydrolyase (DHQase) (EC 4.2.1.10), shikimate oxidoreductase (SORase) (EC 1.1.1.25), shikimate kinase (EC 2.7.1.71) and

chorismate mutase (CM) (EC 5.4.99.5) have been detected in roots of spinach (Spinacia oleracea L.) plants, grown in hydroponics. Three of these enzymes, DHQase, SORase and CM were partially purified.
The activities of DHQase and SORase could not be separated, neither by gel permeation nor by ion exchange chromatography. Both activities may be located on the same polypeptide as it was found for the enzymes from spinach chloroplasts (1). The properties of both activities from roots are similar to those of the chloroplast enzymes (1). However enzymes from both tissues show differences in mobility on a non-denaturating polyacrylamide gel.
Two forms of CM could be separated by ion exchange chromatography. One isoenzyme is nearly inactive in the absence of tryptophan like it was reported for the plastidic CM from spinach leaves (2).
Incorporation studies demonstrate that ^{14}C-labeled shikimate is converted into aromatic amino acids in segments of spinach roots.
Based on these results we suggest that aromatic amino acids required for growth and development of roots are synthezised in situ, rather than imported from leaves. Further studies will show whether the non-photosynthetic tissue of plants principally possesses full autonomy in aromatic amino acid synthesis or if from case to case precursors originating from photosynthetic tissue will be imported by phloem transport. Regulation and sub-cellular localization of the shikimate pathway in roots are under investigation.

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Expression of Subunits of the Chloroplast ATP Synthase in Escherichia coli
Subunit I from Chloroplasts can Complement Subunit b

ATP-Synthases of the F_0F_1 type have a hydrophilic F_1-part and a hydrophobic F_0 part. The structure and subunit composition of the F_1 part is similar for enzymes from different sources (chloroplasts, mitochondria and eubacteria). The F_0-part has different subunit composition, e.g. F_0 from E. coli contains three, from chloroplasts four and from mitochondria at least five different subunits. Moreover the homology of the amino acid sequences is low.

We have investigated, whether it is possible to construct functional F_0-parts with subunits from different organisms. The genes of the CF_0 subunits I, III and IV were transformed to Escherichia coli mutants defective for the corresponding subunits. For subunit I, which contains an intron cDNA was used. The resulting strains were tested for growth on succinate, a substrate that can be used only by oxidative phosphorylation.
Subunit I and IV possess presequences, these were removed by site directed mutagenesis, which introduce a start codon at the start of the mature protein and a new ribosome binding site.

While subunit III and IV are not able to complement subunit c and a, the mature, but not the unprocessed subunit I allows growth of E. coli cells lacking subunit c with nearly the normal rate.
Membranes isolated from the hybrid cells show ATP Hydrolysis and ATP dependent Atebrin quenching in nearly the same way as the wild type.

This result is in some way surprising, since there are only very few identical amino acid residues in both polypeptides. On the other hand, no subunit b with point mutations which is incorporated into F_0 and results in an non functional enzyme has been found, indicating that hardly any specific interactions are involved in the function of the subunit.

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Approaches to the Identification of Ozone-inducible Genes in Picea abies, Pinus silvestris and Fagus silvatica

Seedlings from Scots pine (Pinus silvestris) and Norway spruce (Picea abies) were grown with Ingestad medium in Perlite under continuos light over 5 weeks. Alternatively four-year-old trees of pine and beech (Fagus silvatica) and six-year-old trees of spruce were grown in standard substrates. Prior to ozone treatment the seedlings and trees were preadapted to the climatic conditions of the environmental chambers over several days. The plants were treated with 150 ppb or 250 ppb ozone. After variable time intervals samples were taken from needles, leaves, roots and hypocotyls. Total RNA was isolated using a modificated procedure for trees. Slot blot hybridizations were made with over 25 stress relevant heterologous gene probes. Selected clones were used in Northern blot hybridizations. The results presented on the poster might allow to compare well studied stress responses to ozone effects. To circumvent problems with heterologous probes in future studies we prepared cDNA libraries from poly(A)$^+$-RNA of ozone-treated and control clonal spruce needles. The libraries

are being differentialy screened to systematically isolate ozone induced cDNA clones.

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Lithium uptake in human lymphocytes and erythrocytes

Lymphocytes were isolated from fresh human blood by Ficoll-Paque density gradient centrifugation and stored over night within RPMI-medium pH 7.4. Lymphocytes and erythrocytes from the same donor were suspended in Hanks balanced salt solution, supplemented with EDTA and Lithiumchloride. The effects of Ouabain on the Li^+-uptake in lymphocytes and erythrocytes were studied. At suitable time intervalls the Li^+-uptake of the cells was stopped by washing the cells in Hanks balanced salt solution at 0°C and centrifugation. After lysis of the cells in a mixture of saponin, tricloracetic acid and aq.bidest. the intracellular Li^+ was measured by flamephotometry. The cell volume was measured gravimetrically.

The Li^+-uptake is a first order reaction which can be fitted to an exponential function. The Li^+-uptake in lymphocytes exhibits strong interindividual differences and ranges from $4.1 \cdot 10^{-10}$ - $8.5 \cdot 10^{-10}$ mol/(min·1.0·10^7 cells) at pH 7.4; 37°C; 10 mM extracellular LiCl. By contrast, the initial Li^+-uptake in erythrocytes is in the range of $3.3 \cdot 10^{-13}$ - $8.3 \cdot 10^{-13}$ mol/(min·1.0·10^7 cells). The lithium equilibrium distribution is 0.6±0.17 in lymphocytes (mean ± SD, 7 expts.) and 0.26 ± 0.04 in erythrocytes (mean ± SD, 4 expts.). The cell volume of the lymphocytes amounts 222.85 ± 16.6 μ^3/1.0·10^7 cells (mean ± SD, 6 expts.). In lymphocytes 0.1 mM Ouabain causes an reduction of the Li-uptake of about 68 %; 0.25 mM Ouabain of about 75 %. At 0.1 mM Ouabain the Li-uptake in erythrocytes can be reduced of about 51 %, indicating that a part of the Li-uptake in human lymphocytes and erythrocytes is mediated through the Na^+/K^+-pump.

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Overproduction of the uncI Gene Product of the ATP Synthase of Escherichia coli

The ATP synthase of Escherichia coli catalyses the final step in oxidative phosphorylation. The multimeric enzyme complex is composed of eight different subunits which are encoded by the unc (atp) operon. However, DNA sequence analyses revealed that a ninth gene, uncI (1), exists encoding a hydrophobic and basic protein of unknown function. Expression of the i protein has been observed in vitro (2), but not in intact cells or membrane preparations. Complementation studies (3) and the characterization of an uncI deletion strain (4) showed that this protein is not essential for an active ATP synthase complex. However, compared to the wild type the deletion strain showed a reduction in growth yield.
In order to characterize the uncI gene product and to investigate a putative functional relationship of this protein to the ATP synthase complex, the i protein was overproduced by use of pJLA vectors (5) and purified to homogeneity by chloroform/methanol extraction followed by ion exchange chromatography. Amino acid sequence analysis of the uncI gene products encoded by different plasmids revealed that in contrast to data deduced from DNA sequence analyses (1) the chromosome-encoded i protein contains the N-terminal sequence Ser-Val-Ser-Leu-Val-Ser-Arg and has a molecular weight of 13,504. Immunoblot analyses with polyclonal antibodies against the i protein indicated that the uncI gene product is cosolubilized with the F_o part from membranes of E. coli strain K12. Whether the uncI gene product is associated with the F_o part or only solubilized under the same conditions is under investigation.

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G. Schönknecht, R. Hedrich, W. Junge & K. Raschke

A voltage-dependent chlorid channel in the photosynthetic membrane of a higher plant

During photosynthesis light drives a net uptake of protons into the thylakoid lumen. The resulting electrochemical potential difference of the proton ($\Delta\tilde{\mu}_{H^+}$) provides the energy for ATP synthesis by a proton-translocating ATPsynthase (CF_oCF_1). In the steady state $\Delta\tilde{\mu}_{H^+}$ is mainly determined by ΔpH whereas the electric potential difference (Δψ) is vanishing small. Net H^+ translocation is electrically almost fully compensated by the translocation of other ions. Earlier studies indicated that proton uptake was electrically balanced by Cl^- influx into the thylakoid, or by Mg^{2+} efflux, or by both. The molecular mechanisms involved were not known. During

patch-clamp measurements on osmotically inflated thylakoids of *Peperomia metallica* we observed the activity of a voltage-dependent anion-selective channel[1]. At 30 mM [Cl^-] the single-channel conductance was 65 pS, showing ohmic behaviour between -80 and +80 mV. The opening probability was maximal at about +40 mV (inside the thylakoid). Application of voltage steps caused additional superimposed transient channel openings. We were not able to observe K^+ or Mg^{2+} channels in thylakoid membranes exposed to solutions containing 5 mM $MgCl_2$ and up to 200 mM KCl. Anion flow through this Cl^- channel would allow a rapid, voltage dependent electric compensation of the light-driven proton uptake into the thylakoid lumen.

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Identifizierung funktioneller und struktureller Domänen des E.coli K12 Mannitol-Transportsystems

Struktur und Funktion des Mannitol-spezifischen EnzymII (Mtl-EII) des Phosphoenolpyruvat-abhängigen Kohlenhydrat-Phospho-Transferase-Systems (PTS) wurden unter Verwendung von 3'-terminalen Deletionen des klonierten E.coli K12 mtlA-Gens untersucht (1). Restriktionsanalysen der entstandenen Plasmide, funktionelle Analyse der Deletionsproteine und Immunoblottingexperimente ergaben Hinweise auf drei funktionelle Domänen;
1. Der N-terminale membrangebundene Teil des Mtl-EII entspricht einer Mannitol-spezifischen Pore, die durch Phosphorylierung in eine für die Translokation des Substrats geeignete Konformation überführt wird. Im nicht-phosphorylierten Zustand erfolgt zwar die Bindung nicht aber die Translokation des Substrats.
2. Die notwendigen Phosphorylierungsstellen wurden im C-terminalen, cytoplasmatischen Bereich lokalisiert.
3. Der N-terminale membrangebundene Teil enthält neben der Permeasefunktion auch die Substratbindungsstelle.

Es wurde ein System aufgebaut welches die Isolierung von Porenmutanten erlaubt, die Mannitol auch im unphosphorylierten Zustand ins Cytoplasma translocieren können. Die Verstoffwechslung des freien Mannitols in solchen Porenmutanten erfordert eine entsprechende Dehydrogenase. Hierzu wurde das zugehörige Gen des Arabinitol-Operons aus Klebsiella pneumoniae isoliert und in E.coli K12 exprimiert.
Zur weiteren Charakterisierung der funktionellen Domänen der Mannitol-Permease wird versucht, unterschiedliche Transport- und Phosphorylierungs-Punktmutanten im mtlA Gen zu isolieren, sowie die genaue Kartierung der mtlA-Deletionen durch DNA-Sequenzierung durchzuführen.

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The interaction between trophozoites of Entamoeba histolytica and human extracellular matrix proteins

The parasitic protozoon *Entamoeba histolytica* is the trigger of amebiasis in man, which represents a major health problem in tropical and subtropical countries. Since the trophozoite form of this organism is able to penetrate solid organs, it requires receptors for contacting and hydrolytic enzymes for demaging the host's tissue.
Recently we isolated and characterized the major protease from soluble cell extracts which was able to degrade native human extracellular matrix proteins, such as type I and V collagens and fibronectin, as well as the basement membrane proteins collagen type IV and laminin, respectively [1,2]. Immunohistochemical studies showed that this enzyme was exclusively localized in lysosome-like vesicles of the cells. Furthermore, we found an affinity of intact trophozoites for the attachment to surfaces loaded with human fibronectin and laminin. Since the binding to fibronectin coated surfaces was competed by unbound fibronectin, this effect should represent a specific behaviour. The binding effect of soluble ^{125}J-radiolabeled fibronectin was time-dependent covering a maximum after 15 min at constant fibronectin concentrations, and concentration-dependent reaching a plateau with 160 ng ^{125}J-fibronectin per 10^6 cells after 30 min. Considering that the protease is apparantly not secreted in full scale by the amoebas we suggest that the penetration of the host tissue is preceded by a surface contact of the amoebas via a fibronectin receptor, which in the following is degraded by the protease released after changes in the membrane structure of the amoebas.

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Characterization of chloroplast DNA from the marine microalga Nanochlorum eucaryotum

The small (1.5 µm) unicellular alga Nanochlorum eucaryotum (N.e.)(1) exhibits features that ra-

rely have been observed in green algae[2]. We assume that N.e. is reduced in evolutionary terms. Nucleotide sequence comparisons with the 18SrDNA of N.e. favor evolutionary reduction as the source of its minimal eukaryotic features[3].

The study of the genetic outfit of this organism may deliver valuable informations about the evolution of green algae and their chloroplasts. Since the cell wall of N.e. contains the highly recalcitrant sporopollenin[4], standard methods for the isolation of intact organelles from which the genetic material could be obtained were not applicable. Therefore we extracted the total DNA from N.e. cells.

Nuclear DNA and chloroplast DNA (ctDNA) were then separated from total DNA and purified by repeated CsCl density gradient centrifugation[5]. The AT-rich DNA fraction (34 % GC, 3 % of total DNA) was identified as ctDNA by hybridization with ctDNA probes from spinach. The size was determined to 90 kb by restriction endonuclease digestion and by reassociation kinetics. A library of the ctDNA was constructed by cloning partially digested HindIII and totally digested Cla I fragments in pBR322. By colony hybridization, two consecutive segments of the ct genome (9 kb and 15 kb) were detected. The 9 kb segment carries the genes psb A, rbcL and atp A in an order very different to other ct genomes; in Marchantia polymorpha (M.p.) e.g. these genes are spread over a streach of 50 kb DNA. The 15 kb segment, containing the 16S and 23SrRNA genes, was partially sequenced by the dideoxymethod.

Comparison with the totally sequenced ct genomes of M.p. and Nicotiana tabacum revealed greater similarity of N.e. with M.p., however significant differences were also found. The spacer region between the 16S and 23SrRNA genes is much shorter (400 bp for N.e., 2300 bp for M.p.); furthermore in N.e. the intron of the ala UGC tRNA gene is absent. Construction of a physical map of total ctDNA and taxonomic classification of their rRNA sequences are in progress.

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R. Schubert, P. Prehm

The Hyaluronate Receptor of B6-Cells

We have isolated two receptor proteins from an eukaryotic cell which bound hyaluronate. Hyaluronate (HA) affects cell adhesion, cell motility and cell morphology in vitro and has been implicated in regulating cell behaviour during embryogenesis. Some of these effects are likely to be mediated by interaction with receptors at the cell surface and indeed HA binding proteins have been reported on the surface of different cell types [1][2]. B6-cells synthesize large amounts of hyaluronate as the predominant glycosaminoglucan.

Plasma membranes were isolated from B6-cells and extracted with Triton X114. At 37° C Triton X114 separated into an organic phase containing most membrane proteins and an aqueons phase containing HA and the binding proteins. These were further purified by ion exchange chromatography on DEAE-sephacel to yield two proteins with molecular weights of 100 KD and 60 KD. We elicited polyclonal antibodies against the 100 KD and 60 KD proteins. These antibodies inhibited the binding of HA.

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and A. Schäffer and J. Kägi

Studies on structural and catalytical principles of D-Xylose Isomerase from Streptomyces albus

D-Xylose isomerase (E.C. 5.3.1.5) catalyses the reversible isomerisation of α-D-xylose to α-D-xylulose and α-D-glucose into α-D-fructose. The enzyme from S. albus is a tetramer (M_r 172,500) of four identical subunits with M_r 43,100 as revealed by UV-Laser- Desorption-Mass-Spectroscopy (1).

Upon SDS-treatment the tetramer dissociates into dimers, which are still catalytically active. In consistence with these results the quaternary structure of the tetramer determined by X-ray cristallography has been shown to be a dimer of dimers (2). At higher temperature dissociation into monomers occurs with loss of activity.

The enzyme requires Mg^{2+} for the catalysis, which can be substituted by Co^{2+}. Spectral studies in the visible range indicate two different binding sites per monomer. The higher affinity site (B-site) seems to be octahedral, the lower affinity site (A-site) has a pentacoordinated or distorted tetrahedral structure, as characterized by EAS and MCD. Spectroscopically controlled exchange of Co^{2+} by other cations makes it possible to use different physicochemical studies.

After addition of substrates or inhibitors the Co^{2+} in the A-site changes its geometry to an octahedral structure, however only substrates generates a new absorption band near 320 nm. There is evidence that this absorption is produced by a π-π^*-transition of an intermediate, formed during the isomerisation.

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Jürgen Schumann

Coupling of proton translocation to ATP synthesis and hydrolysis catalyzed by the F_oF_1 ATPase of chloroplasts

Two different, active enzyme conformations were assumed for the proton ATPase (F_oF_1 ATPase) of chloroplasts (1,2):

i) the "energized enzyme form" predominant on energized membranes is able to form ATP from ADP and phosphate; its proton sites face the stroma;

ii) the "deenergized enzyme form" present in the dark after energization catalyzes "light-triggered" ATP hydrolysis; its proton sites face the thylakoid lumen.

Two different conditions allow the conversion between the enzyme forms:

i) the H^+ sites of the enzyme are occupied by three protons, and no catalytic reaction occurs;

ii) the H^+ sites of the enzyme are deprotonated, and the conformational change is accompanied with cleavage/formation of the anhydro bond.

In light-driven ATP synthesis, the energized, deprotonized enzyme binds ADP and phosphate; ATP is formed during the conversion to the deenergized enzyme form. Protonation of the H^+ sites at the inside will then re-establish the energized enzyme form followed by ATP release, deprotonation, and substrate binding.

In light-triggered ATP hydrolysis, the active, deenergized, deprotonated enzyme form binds ATP. Cleavage of ATP establishes the energized enzyme species which will then bind protons from the stroma. The conformational change transfers these protons into the thylakoid lumen thus forming a proton gradient.

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Stephan Schuster and Edmund Baeuerlein

Basal body associated disks as new structural elements of the flagella apparatus of *Wolinella succinogenes*

Bacteria swim by means of the rotation of one or more flagella driven by the electrochemical proton or sodium gradient. Several different types of flagellation are known so far. Enteric bacteria such as *Salmonenella typhimurium* or *Escherichia coli* have several flagella located randomly around the cell body. Other bacteria like *Aquasperillum serpens* or *Halobacterium halobium* are monopolar multiple flagellated with a bundle of flagella forming one superflagellum on ccw rotation. A third type of bacteria is equipped with only one flagellum located at the pole or any other distinct area in the cell wall.
Morphological investigations on the monopolar, single flagellated, Gram-negative, anaerobic bacterium *Wolinella succinogenes* showed an additional disk as a new structural element of the basal body. A similar structure has been found previously in outer membrane preparations of *Aquaspirillum serpens*. With this "intact flagella" preparation it has been shown for the first time, that these concentric membrane rings (CMRs) are more likely related to the basal body, as a part of the flagellar apparatus, than to the outer membrane. The disk, with a diameter of 140 nm or smaller, consists of up to 23 concentric rings arranged around the flagellar rod. The rings are spaced with periods of 33 Å. The diameter of the disk correlates with the number of the rings, smaller disks contain a smaller number of rings. The disks are located between the L and the P ring of the basal body. Thin sections of whole cells, as well as from spheroplasts, show that they are attached on one side to the outer membrane at its periplasmic side. The disk represent a very rigid structure, forming a depression within the cell wall. After dissociation of the flagellar filament, basal bodies with disks could be obtained. Using a new procedure for isolation of the entire disk, we hope to obtain a complete set of disk proteins. Further investigations, concerning image analyzing techniques and in the field of protein chemistry and genetics are necessary to answer the questions about structure and function, as well as whether such basal disks are a common phenomenon or are only found in polar flagellated bacteria.

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Reconstitution of Trimeric Phage P22 Tail Spike Protein

Intermediates in the intracellular chain folding and association pathway of the P22 tailspike endorhamnosidase have been previously identified by physiological and genetic methods [1]. Conditions have now been found for the in vitro refolding of this large (M_r = 215 000) oligomeric protein. Purified Salmonella phage P22 tailspikes, while very stable to urea in neutral solution, were dissociated by moderate concentrations of urea at acidic pH. The tailspike protein was denatured to unfolded polypeptide chains in 6 M urea, pH 3, as disclosed by analytical ultracentrifugation, fluorescence, and circular dichroism. Upon dilution into neutral buffer at 10 °C, the polypeptides spontaneously fold and associate to form trimeric tailspikes with high yield. Like native phage P22 tailspikes, the reconstitution product is resistant to denaturation by dodecyl sulfate in the cold and displays endorhamnosidase activity. Sedimentation coefficients, electrophoretic mobility, and fluorescence

emission maxima of native and reconstituted tailspikes are identical within experimental error.
Observations on reconstitution kinetics suggest close similarities to the in vivo folding and association pathway of P22 tailspikes [2]. Rapid folding is followed by association to detergent-sensitive trimers. From such "protrimers", mature tailspikes are formed in a very slow folding reaction on the trimer level. By characterization of intermediates, localization of temperature-sensitive steps, and analysis of the effect of previously identified folding mutations, the reconstitution system described should allow comparison of in vivo and in vitro folding pathways of this large protein oligomer.

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C. Seehaus und G. Auling

Interferenz von zweiwertigen Ionen mit dem hochaffinen Mn-Transport von Aspergillus niger und Mn-Insensitiven Mutanten

Der Citronensäureproduzent Aspergillus niger hat ein hochaffines, energieabhängiges Mn-Transportsystem [1]. Von Aspergillus niger - Stämmen, die wir als Mn-insensitive Mutanten zur Prüfung erhielten, zeigten einige eine deutlich reduzierte Aufnahme von ^{54}Mn. Um mehr über den Mechanismus der Mn-Aufnahme zu erfahren, sollte das Transportverhalten einer dieser Mutanten genauer charakterisiert werden.

Nachdem eine teilweise Aufhebung der Mn-Insensitivität durch einen Symport mit Citrat bereits nachgewiesen werden konnte [2], wurden die kinetischen Parameter des Mn-Transportes der Mutante bestimmt und mit denen des Wildtyps verglichen.

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J. Seitz, S. Hüntemann and G. Aumüller

Tissue-type transglutaminase in rat testis

Sertoli cells from rat testis are stimulated by FSH (follicle stimulating hormone) that is bound by a specific receptor on the plasma membrane (1). Grasso et al. (2) have demonstrated a stabilization of the hormone-receptor complex by a membrane-associated Ca^{2+}-dependent transglutaminase (TGase) through protein cross-linking. The precise subcellular localization of this TGase is unknown as yet; both an association with plasma membranes and a membrane-adjacent localization in cytoplasm are discussed. We have tried to characterize the enzyme localization by fractionated centrifugation of testis homogenates. The enzyme activity was determined by measuring the incorporation of ^{14}C-putrescine into N,N'-dimethylcaseine at pH 8.5. About 15% of the activity we recovered in a membrane rich fraction which is pelletet after 30 min centrifugation at 20,000g. The enzyme was solubilized by a mixture of KSCN and DTE, as well as by CHAPS and octylglucoside, indicating that it is not an integral membrane protein. Also by treatment with phosphoinositol-specific phospholipase C (PI-PLC) a removal from membranes was achieved. The latter finding indicates the presence of a phosphoinositol anchor (3). The bulk of TGase activity was not precipitated even after 2.5 h centrifugation at 150,000g. A more then 150 times enrichment of the enzyme was achieved using fractionated ammonium sulfate precipitation (50-60% saturation) and gel filtration on a Sephacryl S200 column. A further purification will be achieved with preparative isoelectric focusing in a Rotophor™-system (BioRad), yielding a preparation of homogeneity. A specific polyclonal antibody will be prepared which allows a direct immunological comparison with the membrane-bound isoform and which will be used for immunoelectron microscopic localization of rat testicular TGase at the subcellular level.

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The K^+-Translocating Kdp-ATPase from Escherichia coli: Structure and Function of the Three Kdp Subunits

The Kdp-ATPase from E. coli [1], together with eukaryotic enzymes like the Na^+, K^+-ATPase of the animal plasma membrane or the H^+, K^+-ATPase from gastric mucosa, belongs to the class of K^+-transporting P-type ATPases. Within this group, the bacterial K^+-ATPase is functionally unique because of its high affinity for K^+ (K_m for transport = 2 µM) and its extraordinary high specificity for this ion. These properties reflect the emergency character of the system which is expressed and maximally activated only in the bacteriostatic situation of K^+ limitation. Furthermore, the Kdp-ATPase is of an unusual structural type within this ATPase class due to its composition of three subunits: KdpA, KdpB and KdpC [2].

Our recent working models on the structure and function of the three subunits will be presented. These conceptions are based on (i) theoretical prediction methods for protein structure, (ii) homology comparisons and (iii) experimental data on the phosphorylation[3], topography (proteolytic studies, chemical surface labelling) and the analysis of mutants lacking one of the subunits but expressing and inserting the other two into the membrane.

The data support the hypothesis that KdpB is the energy providing subunit (ATP binding and hydrolysis), KdpA the energy consuming component (K^+ binding and translocation) and KdpC the energy transducing link between them.

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B.Siebers, P.Gräf and E.W.Weiler

Properties of Calcium Channels in the Plasmalemma of *Commelina communis* L.

Tightly sealed, inside-out plasmalemma vesicles can be obtained from leaf microsomes of *Commelina communis* in sufficient yield and high purity using aqueous two-phase partitioning techniques. Primary active loading of these vesicles with $^{45}Ca^{2+}$ can be accomplished in this system exploiting the properties of the Ca^{2+}-ATPase of these vesicles and without interference from the H^+-ATPase of the same vesicles (Gräf and Weiler, Physiol.Plant.(1989) in press). Preloaded vesicles are tightly sealed for $^{45}Ca^{2+}$, but release the loaded radiotracer quickly and completely after addition of trifluoperazine (TFP). Üsing the arsenazo III dye technique, it was shown that freshly prepared plasmalemma vesicles not preloaded with Ca^{2+} release, upon addition of TFP, large amounts of membrane-associated Ca^{2+}. CAM did not antagonize the effect of TFP on $^{45}Ca^{2+}$ release. TFP-induced release of $^{45}Ca^{2+}$ can be blocked efficiently with La^{3+}. The properties of the La^{3+}-sensitive efflux system, activated by TFP-induced removal of membrane-bound endogenous Ca^{2+}, were studied in detail and will be given. The results are consistent with the presence of a Ca^{2+}-inactivated, La^{3+}-sensitive channel which directs influx of Ca^{2+} into the plant cell, in the plasma membrane of *C.communis*.

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M. Speth and H.-U. Schulze

Inhibition of Hepatic Glucose-6-Phosphate : Phosphohydrolase by Methylthioadenosine

The glucose-6-phosphate: phosphohydrolase is an integral protein of the microsomal and nuclear membrane which is deeply embedded within the hydrophobic part of the bilayer. In terms of the conformation-substrate transport concept (1) the phosphohydrolase traverses the microsomal membrane as a channel-protein bearing the catalytic part of the enzyme. In our present studies we have investigated the effects of methylthioadenosine (MTA) , a by-product of hepatic polyamine synthesis and potent inhibitor of cell proliferation, on glucose-6-phosphate hydrolysis of rat liver microsomes. Incubation of native microsomes with MTA at 37°C shows that glucose-6-phosphatase activity at 37°C is progressively inhibited but enzyme activity of the same microsomes assayed at 0°C is not affected. Subsequent modification of these MTA-treated microsomes with Triton X-114, however, reveals that glucose-6-phosphatase activity at 0°C as well as at 37°C is inhibited by the reagent. Furthermore, the data obtained from competition studies between MTA and the anion transport inhibitor DIDS on native microsomes suggest that MTA interacts with the DIDS binding site at the phosphohydrolase which is directly accessible from the cytoplasmic membrane surface (2). From these results we conclude that MTA inhibits microsomal glucose-6-phosphate hydrolysis by direct interaction with the integral phosphohydrolase of the native membrane. The temperature-dependent effect of MTA on glucose-6-phosphatase of native microsomes is interpreted in terms of different enzyme conformation forms elicited by the surrounding membrane at 0°C or 37°C.

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M.L. Sprengart and E. Fuchs

Possible interaction of mRNA sequences at the beginning of a gene with the 16srRNA during initiation of translation in *E. coli*

The signal for the initiation of translation in E.coli, the ribosome binding site (RBS), is thought to consist of an initiation codon and a Shine-Dalgarno sequence separated by a suitable spacing. These domains, however, are not sufficient to define an efficient RBS (1). Sequences further upstream and downstream of this area make strong contributions to its activity (2). We cloned the bacteriophage T7 gene 0.3 RBS on DNA fragments of different length in an expression vector (3) just upstream of the mouse dihydrofolate reductase gene to control the translation of its sequence in both, in vivo and in vitro experiments. Downstream sequences between +9 and +37 nucleotides of the T7 0.3 gene increased the RBS activity by almost two orders of magnitude. Most efficient was a sequence between +15 and +30 nucleotides of the

gene. This sequence shows a complementarity to nucleotides number 1471 - 1482 of the 16srRNA. This rRNA region is bound into a weak secondary structure in the ribosome which could be opened by the action of the S1 ribosomal protein. Similar comlementary sequences to this rRNA region exist about +4 to +25 nucleotides downstream of the initiation codon in other efficient RBS's of the E. coli genome and that of its bacteriophages. We therefore suggest that this region specifies a further stimulatory interaction between mRNA and 16srRNA besides the Shine-Dalgarno interaction in the translational initiation process.

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K. Stadtlander

Testing the sensitivity of the surface uptake system of Tetrahymena towards environmentally hazardous chemicals

In literature there are two systems described for Tetrahymena for the uptake of solutes and particles(1). They are called the oral uptake system (OUS) and the surface uptake system (SUS). When the further is blocked by cytochalasin B and / or cycloheximid the cells have the surface uptake system left. Then growth is accomplished very well only in a synthetic medium without proteins but with aminoacids, which does not induce the formation of food vacuoles, so the SUS is the only way of food intake. To get a closer look to this uptake system, I tried to characterize the SUS under conditions, where food uptake via endocytosis is not possible. First experiments dealt with the uptake of guanine, because it is well known that it is essential, Tetrahymena requires a source for a purine ring system. The experiments failed because of lack of standardization, so I changed to the guanine nucleoside, guanosine. These results are presented .
Furthermore it is of great interest to mankind to find out suitable living systems to test for chemical hazards. In order to try to establish a test system in analogy to our Yeast-cell test system (2), which was developed to reduce and/or replace testing with higher organized animals, there are experiments with Tetrahymena in progres in our group . This organism has great advantages over yeast cells because of its ability to move, to react via chemokinesis when chemicals are introduced into the surrounding medium. Furthermore it has a certain comunication system using Calcium-ions and has some receptors for hormones which sense is still under investigation. These cells are up to ten times more sensitive to chemicalsas preliminary tests have shown, which is the biggest advantage when comparing with yeast.

Five environmentally hazardous chemicals were chosen in cooperation with the German Federal Environmental Agency. The potential noxious effect of these chemicals on the uptake of guanosin was measured. The results are presented.

More detailed work is in progress with some other chemicals on other biochemically measurable functions of the cells, as there is the chemokinesis, electrorotation of whole cells, changes of Ca - fluxes within the cells as a response to environmental changes and the energy charge and/or the oxygen consumption of the cells during growth in the presence of water-soluble chemicals.

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K.Stapel, S.Singh, D.P.Agarwal and H.W.Goedde

In Vitro Import of the Human Aldehyde Dehydrogenase 2 Precursor into Rat Liver Mitochondria

The human mitochondrial aldehyde dehydrogenase (ALDH2)(EC 1.2.1.3.) a tetramer of four idemtical subunits is coded by a nuclear Gene assigned to chromosome 12. As deduced from the full length cDNA sequence(1), the subunits are synthesized as larger precursors which include a N-terminal leader peptide of 17 amino acids. The amino acid composition of this leader peptide shares the typical features with most other mitochondrial import signals: amphiphilicity, rich in basic and hydroxylated amimo acids and no acidic amino acids.

In the present study the import of in vitro synthesized ^{35}S-labelled precursor polypeptides of ALDH2 into isolated rat liver mitochondria was demonstrated by an in vitro import assay system. Characterisation of the import process was carried out by SDS PAGE of the supernatant and mitochondrial pellet derived from the assay mixture and subsequent autoradiography. The cleavage of the leader peptide occurs simultaneously with the import, as only mature subunits are found in the mitochondrial pellet fraction. After modification of the cDNA by "Site Directed Mutagenesis", the mature subunits of ALDH2 were synthesized in vitro. The modified subunits were found not to be imported into mitochondria under conditions used for the import of the precursor peptides.

Possible influence of factors such as electrochemical gradient across the mitochondrial membranes, ATP requirement, and the conformation of ALDH2 prior to and after the import, are under investigation.

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N.J. Stapper, W. Staib, and P. Schadewaldt

α-Adrenoreceptor mediated production of inositol phosphate isomers in skeletal muscle fibres of rat

In skeletal muscle, α-adrenergic agonists evoke a rapid [^{3}H]-inositol phosphate accumulation in the presence of lithium. [^{3}H]Phosphoinositide content, however, remains unchanged (1). In an attempt to unravel the nature of the precursor phospholipid, we examined α-adrenergic agonist induced changes in the pattern of skeletal muscle inositol (poly)phosphate isomers.

[^{3}H]inositol-prelabelled muscle fibres were prepared from flexor digitorum brevis muscles and separated from mononucleate cells (1). After incubation in the presence of 10mM LiCl, accumulated [^{3}H]inositol (poly)phosphate isomers were separated by HPLC essentially as given in (2). Results are means ± SD (in dpm per 10^3 fibers).

Significant amounts of radioactivity were recovered in [^{3}H]-Ins 1-P (1P), [^{3}H]Ins 4-P (4P), [^{3}H]Ins 1,4-P_2 (IP_2), and [^{3}H]Ins 1,4,5-P_3 (IP_3). 1P and 4P content of fibres amounted to 42 ± 6 and 48 ± 9, repectively. During 30 min of incubation, both isomers accumulated linear with time. In the absence and presence of 10 μM epinephrine, 2-3-fold and 8-10-fold increased levels were reached, repectively. IP_2 content (26 ± 4) remained unchanged in controls but was increased up to 5-fold within 10 min by hormone treatment. IP_3 levels remained essentially unchanged, irrespective of incubation condition. When authentic IP_3 was degraded in saponified fibres, only IP_2, 4P, and (without LiCl) free inositol appeared as products. Preliminary results furthermore indicate that epinephrine treatment of fibres causes a decrease in total IP_3 content but an increase in its specific radioactivity (collaboration G.W. Mayr).

The data suggest that in skeletal muscle the hydrolysis of both phosphatidylinositol (PtdIns) and PtdIns 4,5-P_2 is specifically increased by epinephrine treatment. Unchanged levels of labelled IP_3 may be explained by an increased flux through an IP_3 pool.

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K. Steube, B. Chaudhuri, and J. Heim

Signal processing, precursor-glycosylation and secretion of recombinant human insulin-like growth factor I (IGFI) in Saccharomyces cerevisiae

To study the importance of particular signal sequences for expression, posttranslational modification and secretion of a heterologous protein in yeast, human insulin-like growth factor I (hIGFI) was used as a model system. The sequence coding for mature human IGFI was chemically synthesized (kindly provided by Chiron Corp. Emeryville, USA), inserted into the 2μ-derived multicopy plasmid pDP34 and transformed into appropriate yeast host strains.

To allow for secretion, acid phosphatase (17aa) and invertase (19aa) signal sequences or the α-factor leader (85aa) were fused 5′ to the mature IGFI sequence.

Synthesis and secretion of IGFI was analysed either by pulse-chase experiments, followed by SDS-PAGE or by Western blotting. It turned out that the choice of the signal sequence was very important for efficient processing, secretion and resistance against proteolytic degradation. When α-factor leader was used as a signal sequence, IGFI was synthesized via high molecular weight glycosylated precursors which were processed into mature IGFI within the yeast secretory pathway. Processing and intracellular transit time was rapid, secretion into the medium, however, was considerably slower leading to accumulation of processed IGFI molecules inside the cells.

Use of the acid phosphatase or invertase signal sequences or α-factor leader variants mutated in the vicinity of the processing site(s), led to accumulation of unprocessed precursors inside the cells and a severe impairment of secretion

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A ΔpH clamp technique for measurements of reactions of the proton-translocating ATPase in chloroplasts

Steady state $\Delta\tilde{\mu}_{H^+}$ across the thylakoid membrane is a complex function of light-dependent photosynthetic electron flow, H^+ transport-coupled formation and hydrolysis of ATP as well as non-productive H^+ efflux.

Alteration of one of these parameters necessarily changes the magnitude of $\Delta\tilde{\mu}_{H^+}$. The precise determination of kinetic constants of the H^+-translocating ATPase requires measurements at controlled $\Delta\tilde{\mu}_{H^+}$. For this purpose a ΔpH clamp technique was developed. The principle of the method is based on compensation of accelerated or decelerated H^+ efflux by a corresponding change of H^+ influx achieved by fast electronic regulation of the intensity of actinic light.

As a measure of ΔpH (at $\Delta\psi$ = 0) the calibrated 9-aminoacridine fluorescence signal[1] is employed. The device permits determinations of substrate affinities of the H^+-ATPase and reaction rates at any desired $\Delta\tilde{\mu}_{H^+}$.

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Effect of Lutropin and Follitropin on 3-Hydroxysteroid: NAD(P) Oxidoreductases in Rat Liver Cell Cultures

5α- and 5β-dihydrotestosterone are metabolized in the rat by reduction of the 3-oxo-group. 3α-Hydroxysteroid:NAD(P) oxidoreductases (EC 1.1.1.50) and 3β-hydroxysteroid: NAD(P) oxidoreductases (EC 1.1.1.51) are able to use NADH or NADPH als coenzymes. We have measured the effects of lutropin and follitropin in combination with various steroid hormones on the catalytic activity of 3α-hydroxysteroid:NAD(P) oxidoreductases. The cells were prepared by perfusion of rat livers (female Wistar rats, weight 200-300 g) with collagenase solution using a modification of Seglen's method[1]. 4 x 10^8 cells were transferred to each culture flask containing the culture medium and the hormone combination. Incubation was carried out for 4 days. The cells were then homogenized in 0.3 M potassium phosphate buffer and the enzyme activities were measured as already described[2].

The enzyme reacted to physiologic concentrations of lutropin and follitropin only after 96 hours of incubation by an increased activity (45% and 28%, respectively). Combinations of lutropin and testosterone, or its metabolite 5α-dihydrotestosterone decreased the lutropin effect. 5β-Dihydrotestosterone seemed to augment the effect of lutropin. Similar observations were made with the combination of follitropin and testosterone or its 5α- or 5β-dihydrometabolites. In addition, it was shown by use of radioactive steroids, that the steroids were taken up by the cells from the medium and were metabolized in the cell compartments.

F.Titgemeyer, R.Eisermann, D.Kohlbrecher, W.Hengstenberg and J.W.Lengeler

Amplification of related ptsH genes with the polymerase chain reaction (PCR)

The ptsH genes from E.coli strains HB101, TG1 and a related enterobacterium Klebsiella pneumoniae strain amplified using the PCR technique with genomic DNA as template.
PCR is based on repeated cycles of DNA denaturation, primer annealing and DNA polymerisation[1,2]. Here we present i) the amplification of ptsH from the K.pneumoniae strain KAY2026 using E.coli specific primers[3] and ii) a minipreparation of chromosomal DNA suitable for PCR[4].
The amplification assay with Klebsiella DNA provided the expected 333bp product. This fragment was subcloned and sequenced. The deduced amino acid sequence shows only a single substitution (L63I) to the corresponding HPr of E.coli and S.typhimurium[5].
In a second approach we have developed a rapid minipreparation of chromosomal bacterial DNA to prepare the amplification product as fast as possible[4].
These data suggest the application of the PCR technique for rapid cloning of highly conserved genes like ptsH from a number of near related bacteria species. Further investigations to study structure, function and evolution of HPr in other gram negative organisms are in progress.

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Translational coupling in the sucrose operon of *Klebsiella pneumoniae*

The sucrose regulon of *Klebsiella pneumoniae* consists of at least five genes termed *scrKYABR*. Transport is directed by a sugar specific pore (*scrY*) and the phosphoenol-pyruvate dependent carbohydrate: phospho-transferase system (PTS) Enzyme II^{Scr}/III^{Glc} (*scrA/crr*). Hydrolysis (Invertase, *scrB*) and fructose phosphorylation (Sucrose Kinase *scrK*) complete the peripher metabolism. The operon is negative regulated by a repressor (*scrR*).
We cloned a 3.7kb fragment, comprising the *scr'YABR'* region. Several mutants lacking II^{Scr}, invertase or all enzyme activities(1) were mapped by recombination analysis. Surprisingly all invertase inactivation mutations mapped in the structural gene for scrA. These mutations also reduced II^{Scr} function, probably because of a missense mutation with polar effect on the translation of *scrB*. Other *scrA* mutantions allowed normal invertase expression. In addition *scrAB* showed overlapping stop and start codons. These results indicate a translational coupling(2) between *scrA* and *scrB*.
Another interesting mutant not inducible by known inducers for any of the sucrose enzymes also carries a mutation in *scrA*. Further investigations showed the presence of a second mutation resulting in the constitutive expression of the pts-dependent fructose PTS. Induction by fructose seems to be abolished by fast degradation of internal inducer in this mutant. These experiments corroberates the previous hypothesis(3), intracellular fructose is the natural inducer of the pts sucrose pathway in *Klebsiella pneumoniae*.

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B.Trinczek, C.Bode and G.Schwoch

Immunolocalization of cAMP-dependent Protein Kinase Subunits in Mitochondria from Various Rat Tissues

Although an influence of cAMP on mitochondrial functions has been repeatedly shown, it is still controversial whether cAMP-dependent protein kinases are present in mammalian mitochondria. Using specific antisera against their catalytic (C) and regulatory (RI and RII) subunits in an immunogold procedure, we localized the cAMP-dependent protein kinases in mitochondria from cells of liver, kidney, parotid gland, pancreas, testes, heart and skeletal muscle. In all tissues examined, the labelling density in the mitochondria was remarkably high when compared with that in the cytoplasm and in the nucleus. The localization of gold particles indicated a presence of the cAMP-dependent protein kinases at the inner membrane-matrix space of the mitochondria. These immunocytochemical findings were confirmed by immunochemical measurements with fractionated broken tissue. Our results indicate a direct role of cAMP-dependent protein kinases in the regulation of mitochondrial processes which are localized in the inner membrane/matrix compartments of the organelle.

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Ingo Trompeter, Inge A.Brand and Hans-Dieter Söling

Primary Sequence of a New Zink-binding Protein from Rat Liver

Brand and Söling (1) have recently described a rat liver protein which leads in a Zn^{2+}-dependent way to the disssociation and reversible inactivation of phosphofructokinase-1 (E.C. 2.7.1.11). This protein is characterized by a complete lack of aromatic amino acids and a 45% content of acid amino acids.
On the basis of partial amino acid sequences reported in (2), oligonucleotides were synthetized and used for the detection of appropriate cDNA clones from a gt11-rat liver library. The full primary sequence of the Zn^{2+}-binding protein could be deduced from a 936 bp clone.
In contrast to the results of SDS-PAGE which gave an estimated M_r of 19,000, the primary sequence gave a M_r of 11,400. This result was confirmed by Laser-time-of-flight mass spectrometry.
One partial peptide analyzed previously could not be detected in the identified protein sequence. This observation together with the fact that the purified protein appears always as a double band inspite of homologeous regions argues for the existence of isoproteins.

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Maximilian Tropschug, Ilse Barthelmess, Klaus Dietmeier and Walter Neupert

Cyclosporin A-binding Protein (Cyclophilin) mediates Cyclosporin Action in Saccharomyces cerevisiae and Neurospora crassa

Cyclosporin A (CsA) is a potent immunosuppressive undecapeptide which originally was isolated as an antifungal antibiotic, killing fungi like Neurospora crassa and certain strains of yeast. Neither the target of the immunosuppression nor the biochemical mechanism of the cytotoxic effect are known. An ubiquitous cytosolic protein (cyclophilin) was isolated from bovine thymus and human spleen which is able to bind CsA with high affinity (1) and which was postulated to be the target of CsA action. Recently, it was shown that cyclophilin is identical to peptidyl prolyl cis-trans isomerase, an enzyme which is able to accelerate the slow folding phase of certain proteins in vitro (2,3).
We use the lower eukaryotes Saccharomyces cerevisiae and Neurospora crassa as model systems to unravel the mechanism of CsA action. Cyclophilin is located in N. crassa in both cytosol and mitochondria, both forms being encoded by one single nuclear gene (4). Using CsA-resistant mutants of N. crassa and S. cerevisiae with alterations in the cyclophilin proteins, we show that cyclophilin is indeed the target of the cytotoxic effect of CsA. In addition, a cytosolic form of yeast cyclophilin was cloned and is shown to be homologous to human, bovine, rat and N. crassa cyclophilins and to the Drosophila ninaA gene product required for visual transduction (5,6).

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V. Truniger und G. Sweet

Das *glpFK*-Operon und die Permeabilität der Cytoplasmamembran von *Escherichia coli*

Shigella flexneri kann auf Glyzerin als Kohlenstoffquelle wachsen, es aber bei geringer Konzentration im Aussenmedium nicht aufnehmen. Mit dem *glpK*-Gen (Glyzerin-Kinase) cotransduzierbare *Shigella*-DNA wurde mit Hilfe des P1-Phagen ins *E.coli*-Chromosom transduziert, was zu einem Defekt in der Glyzerinaufnahme führt. Lange Zeit wurde angenommen, daß der *Shigella*-Phänotyp in *E.coli* durch eine Mutation im Gen für das Glyzerinfacilitator-Protein (*glpF*) hervorgerufen wird. Wir konnten jedoch zeigen, daß sich durch Einbringen der *Shigella*-DNA der Innenmembran-Aufbau des *E.coli*-Stamms verändert. Seine Membran wird rigider und ihre Permeabilität ist reduziert.
Southern Blot Analyse zeigte einen Unterschied zwischen Hybrid- und Wildtyp-chromosomaler DNA direkt hinter dem *glpK*-Gen. *glpF*- und *glpK*-Mutanten weisen auch eine reduzierte Permeabilität und eine rigidere Membran auf. Scheinbar liegt distal zum *glpK*-Gen im *glpFK*-Operon von *E.coli* ein Gen, das die Permeabilität der Innenmembran reguliert. Durch Verminderung des Cardiolipin-Gehalts der Membran eines *E.coli*-Stamms mit integrierter *Shigella*-DNA steigt ihre Permeabilität und der Glyzerintransport wieder an. Das GlpF-Protein reagiert auf Veränderung der Lipid-Zusammensetzung besonders sensitiv. Wir konnten zeigen, daß sich durch Insertion der *Shigella*-DNA in einen *E.coli*-Stamm die Phospholipidverteilung seiner Membran nicht verändert. Er wird aber resistent gegen Cerulenin, was auf eine Änderung in der Fettsäuren-Zusammensetzung hinweist. Im Phospholipid-Stoffwechsel werden die Fettsäuren an das Glyzerin-3-Phosphat (G3P) angehängt. Ein Zusammenhang zwischen der Glyzerin-Aufnahme, dem G3P-Spiegel in der Zelle und der Regulation der Fettsäuren-Kettenlänge ist gut denkbar.

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ATP Synthesis Catalyzed by the ATP-Synthase from Rdb. capsulata

Chromatophores have been prepared from Rdb. capsulata. The chromatophores were energized artificially by an acid base jump which generates a transmembrane ΔpH and $\Delta\Psi$. The rate of ATP synthesis was measured with rapid mixing techniques. After energization a linear increase of ATP synthesis with reaction time is observed for about 1 s. From the slope of these curves the rate of ATP synthesis is calculated. Rate maximums of about 70 mM ATP/(M BChl·s) are observed up to now. After 1 s reaction time a further increase of the ATP concentration is observed, which is due to an adenylate kinase catalyzed reaction. The data were corrected for this background reaction. The rate of ATP synthesis was measured as a function of ΔpH (at constant pH_{out} = 8.5) and of $\Delta\Psi$.

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Modulation of Transport of mRNA from Nucleus to Cytoplasm by Nuclear Binding of Insulin and EGF

Efflux of rapidly labeled poly(A)-containing mRNA from isolated rat liver nuclei was found to

display a biphasic but opposite response to insulin and epidermal growth factor (EGF). At 10 pM insulin and 1 pM EGF maximal stimulation of transport rate by insulin (to 137%) and maximal inhibition by EGF (to 69%) were obtained. The modulation of nuclear efflux of mRNA occurs at the level of translocation through the nuclear pore, as demonstrated in experiments using mRNA entrapped into closed nuclear envelope (NE) vesicles. The NE nucleoside triphosphatase (NTPase; EC 3.6.1.15) activity, which is thought to mediate nucleocytoplasmic transport of at least some mRNAs, responded to insulin and EGF in a manner paralleling that found for mRNA transport rate. Investigating the effects of the two growth factors on transport of specific mRNAs, poly(A)-containing actin mRNA was found to display the same alteration in efflux rate as rapidly labeled, total poly(A)-containing mRNA. In contrast, efflux of histone H4 mRNA, which lacks a 3'poly(A) sequence, decreased in response to insulin, reaching minimum levels at that time at which maximum levels of actin mRNA transport rate were obtained. Insulin was found to cause an enhancement of NE associated phosphoprotein phosphatase activity and, hence, an apparent inhibition of NE protein kinase activity, resulting in a decrease in poly(A) binding affinity of the phosphorylatable mRNA binding site (=mRNA carrier) within the envelope. EGF, on the other hand, stimulated the protein kinase, which phosphorylates the carrier, and, hence, increased the NE poly(A) binding affinity. Because the stage of phosphorylation of the mRNA carrier, which is coupled with the NTPase within the intact NE structure, is inversely correlated with the activity of this enzyme, an enhancement of poly(A)-containing mRNA transport rate by insulin and an inhibition by EGF occur.

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Claus Urbanke and Achim Schaper

Kinetics of association of single-stranded DNA binding protein from *E. coli* to natural and synthetic polymeric nucleic acids

The single-stranded DNA binding protein from *E. coli* (*E. coli* SSB) plays a vital role in replication, recombination and repair processes. It binds strongly and co-operatively to all kinds of single-stranded nucleic acids. In its physiological function in e.g. the replication process, *E. coli* SSB must bind to the single-stranded DNA (ssDNA) produced by the helicase to protect these single-stranded regions from nuclease digestion and to prevent formation of intramolecular helices. At the same time this binding should not interfere with other processes like e.g. the polymerase reaction.

We tried to measure the rate at which *E. coli* SSB is able to bind to its substrate. Binding to poly(dT) had been shown to be nearly diffusion controlled but due to the apparent negative cooperativity this rate could only be observed at a large excess of DNA over protein. The apparent negative cooperativity in binding of a multidentate ligand to a linear lattice is caused by the fact that two ligands will block a possible third binding site if they do not bind adjacent to each other. Its equilibrium theory has been described in great detail [1],[2] but up to now no complete kinetic theory exists. Forming of co-operative clusters would diminish the apparent negative cooperativity. We could show in stopped flow experiments with free and pre-saturated poly(dT) that the formation of cooperative clusters is faster than the bimolecular association rates. This indicates, that *E. coli* SSB once bound to poly(dT) is free to move along the polymer.

For M13mp8 ssDNA the association rate is three to four orders of magnitude slower. This astonishing observation can be explained by the large content of secondary base paired structure present in the substrate. M13mp8 ssDNA denatured by glyoxal treatment shows similar association rates as poly(dT). Therefore, *E. coli* SSB cannot melt double stranded regions in an active process but rather must wait for transiently opened helices and thereby binding is a very slow process. For the physiological functions of *E. coli* SSB this means that:

1.) *E. coli* SSB is not able to remove existing base paired structures in reasonable time.

2.) *E. coli* SSB cannot melt out helices base by base and thus overcome the high stability of DNA double helices caused by their cooperative structure

3). *E. coli* SSB binding to ssDNA must occur immediately after the double helix has been unwound.

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Polarized expression of polyamine transporters in the LLC-PK$_1$ renal established cell line

Intracellular polyamines are essential for cellular growth and development. The cellular requirement for polyamines can be fulfilled by de novo synthesis and by import through specific polyamine transporters [1]. We have found two different saturable polyamine uptake pathways in LLC-PK$_1$ cells [2]. Both pathways are strongly upregulated when cells are treated with DFMO*, a specific inhibitor of the polyamine synthesis. One of the pathways is Na^+-dependent and has a relatively high affinity for putrescine (K_m = 4.7 μM) whereas the second pathway is Na^+-independent and has a lower affinity (K_m = 29.8 μM)

We have measured polyamine uptake in LLC-PK$_1$ monolayers grown on a permeable support (Millicell HA, Millipore), separating an 'apical' and a 'basolateral' compartment. It was found that polyamine uptake by the Na^+-dependent pathway was preferentially localized at the basolateral cell side. Putrescine uptake from the apical compartment was only 20 - 30 % of the uptake from the basolateral compartment. A similar behaviour was found for spermidine and spermine as substrates. Uptake by the Na^+-independent carrier on the other hand was non-polarized and was equally important when measured from the apical or basolateral compartment.

These data suggest that active transcellular transport from the basolateral to the apical cell side is possible.

*DFMO : DL-2-difluoromethylornithine was kindly provided by Dr. C. D. Houldsworth (Merrel Dow Research Institute, Strasbourg, France)

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Transient Phase and Steady State Kinetics of Monocyclic Cascades

Many regulation processes exist in which a protein is reversibly and covalently modified by an enzymic transfer and removal of a modifying group (1,2). These reactions are catalyzed by converter enzymes which, in turn arise from a previous activation effectors (1). Both the reactions of modification and their reversal form a monocyclic cascade system.

Until now only the steady state of the cyclic cascades has been studied (2). In this communication, we present a kinetic analysis of the whole course of the reaction and therefore, for both the transient phase and the steady state. The kinetic equations for the steady state are obtained as particular cases of the corresponding ones to the transient phase. Moreover, the assumptions made for this kinetic analysis are less restrictive as those made in the literature for the steady state and, therefore, the results corresponding to this state obtained by us are *not* exactly the same as those the obtained by other authors (2).

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Inhibition of Sodium Pumps of *Xenopus* Oocytes by Stimulation of Protein Kinase C.

Full-grown oocytes of *Xenopus laevis* were treated with 50 nM of the phorbol ester PMA (phorbol 12-myristate 13-acetate). Effects of PMA on membrane currents and on ouabain binding were investigated.

During application of PMA, membrane conductance gradually decreased reaching a steady state after about 60 min. While in untreated oocytes a large portion of the membrane current could be inhibited by 10 µM strophantidine, the current remaining in PMA-treated oocytes was insensitive to strophantidine. This demonstrates that application of PMA leads to inhibition of pump current. 50 nM 4α-PDD (4α-phorbol 12,13-didecanoate) also reduced part of total membrane current but the component sensitive to strophantidine was unaffected.

To examine the mechanism of pump inhibition, we estimated the number of pump molecules in the plasma membrane by measuring [^{3}H]ouabain binding capacity. Within 30 min of exposure to 50 nM PMA, the capacity of intact cells to bind ouabain decreased by 70-80%; 4α-PDD had no effect. Permeabilization by digitonin plus 0.02% SDS rendered all sodium pumps present prior to PMA-treatment accessible for ouabain.

The findings suggest that the sodium pump is inhibited by stimulation of protein kinase C, and that this inhibition is brought about by an internalization of pump molecules as has been demonstrated (1) to occur during meiotic maturation.

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Free Solution Isoelectric Focusing of Human Erythrocyte Uroporphyrinogen I Synthase on a Preparative Scale

Uroporphyrinogen I (URO-I) synthase (porphobilinogen ammonia-lyase (polymerizing)) (EC 4.3.1.8) catalyzes the head-to-tail condensation of 4 molecules of the monopyrrole, porphobilinogen (PBG) to form the linear tetrapyrrole hydroxymethylbilane which then cyclizes spontaneously to URO-I. In conjunction with uroporphyrinogen III cosynthase the asymmetric stereoisomer and heme precursor uroporphyrinogen III is produced.

In this report a preparative free solution electrofocusing method for URO-I synthase in an ampholine pH-gradient is described. Partial purification of the enzyme was achieved in a 4 h focusing run. Separation capability of the method for the isozymes occurring as stable enzyme-substrate (mono-, di-, tri-, tetrapyrrole) intermediates was controlled by electrofocusing on ultrathin polyacrylamide gels. Complete separation of the most cathodal isozyme could be obtained in this single step procedure. The examination of these intermediates is of particular interest with respect to the URO-I synthase deficiency in families with AIP. This defect is inherited as an autosomal dominant trait. Electrofocusing is, in this regard, an useful approach to compare normal isozyme patterns with those of URO-I synthase deficient AIP patients and is also an effective tool in detecting new altered structural variants of the enzyme. In contrast to the normal isozyme set with seven isozyme bands, the

fluorescence of the three cathodal bands and the second anodal band was greatly reduced, whereas the intermediate forms showed increased fluorescence intensity.

The significant advantage of this method over the traditional procedures performed in flat beds of granulated gels or in columns containing density gradients, is the rapid collection and prevention of eventual mixing of the fractions. The proteins do not need to be eluted from a solid gel matrix and could be refocused in a narrow pH-gradient immediately (1).

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Genomic Organization of the Human α_1-Microglobulin-Bikunin Gene

Bikunin[1] is the inhibitor subunit of inter-α-trypsin inhibitor (ITI) and is considered as an endothelial cell growth factor. α_1-Microglobulin belongs to the widespread α_{2u}-globulin-superfamily of transport proteins. For both serum proteins, bikunin as well as α_1-microglobulin, an immunosuppressive action has been observed. Both proteins result from proteolytic processing of a common primary translation product[2].

In order to gain some insight into possible structural and functional relationships between bikunin and α_1-microglobulin and to clear their possible roles as acute-phase proteins, we studied the genomic organization of their gene. Genomic clones covering about 35 kb were isolated and characterized by restriction mapping, hybridization experiments and sequencing. By comparison with the cDNA data[2,3] we could establish the complete exon-/intron-structure as well as large sequence regions upstream and downstream of the gene. This allows initial studies of the regulation of its transcription.

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Isolierung des Glycerinfacilitators, GlpF, aus *Escherichia coli*.

Der Glycerinfacilitator ist bekannt als das einzige Beispiel eines Transportproteins, das den Vorgang der erleichterten Diffusion über die *E. coli* Innenmembran katalysiert. Das Gen, *glpF*, das für das Glycerintransportprotein kodiert, ist in der 88min. Region des *E. coli* Chromosoms in einem Operon mit *glpK*, dem Strukturgen für die Glycerinkinase, lokalisiert.

Die Klonierung des *glpF* Gens in ein Plasmid des T7-RNA-Polymerase/Promotor Systems erlaubte die selektive und kontrollierte Überproduktion des in hoher Kopienzahl für die Zelle letalen Genproduktes. Durch diese Überproduktion wurde es möglich, GlpF auf SDS-Polyacrylamid Gelen durch Färbung mit Coomassie Brilliant Blue nachzuweisen. Das aus den Gelen bestimmte Molekulargewicht weicht mit rund 25 000 deutlich von dem aus der Sequenz bestimmten, 29 727 D, ab.

Eine Zellfraktionierung durch osmotische Lyse bildete den ersten Reinigungsschritt. Hierbei wurde GlpF in der Membranfraktion lokalisiert. Durch Anwendung des Trenneigenschaften aufweisenden Detergenz Triton X-114 konnte GlpF als stark hydrophobes Protein klassifiziert werden. Dies korreliert sowohl mit der Lokalisation des Proteins als auch mit Resultaten aus Hydrophobizitäts-Plots. In Anwesenheit von Glycerin zeigt angereinigtes GlpF eine Konformationsänderung. Fortschritte in der säulenchromatographischen Reinigung des Lubrol PX solubilisierten GlpF-Proteins werden beschrieben.

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Influence of oleic acid on Na^+-dependent L-glutamate, succinate and citrate transport in rat renal brush-border membrane vesicles (BBMV)

Recently we showed [1] that cis-unsaturated long chain fatty acids, e.g. oleic acid (OA), inhibited Na^+-dependent D-glucose transport. In the present study we investigated the effects of OA on other Na^+-dependent co-transport systems in BBMV.

Uptake of 100 µmol L-glutamate was measured in the presence of an inward directed Na^+ gradient (Na_o=100 mM) and an outward directed K^+ gradient (K_i=20 mM). At 30 s uptake averaged 2942 ± 371 pmol/mg protein. Peak uptake was obtained at 120 s. 500 µmol OA inhibited glutamate uptake by 7, 10 and 25% at 10, 30 and 60 s (n=7-8), respectively. Inhibition increased to 60% at a concentration of 2 mM OA (30 s). Kinetic analyses (high affinity system) revealed a mixed-type inhibition with a decrease of both Km (from 20 µmol to 8 µmol) and Vmax (2696 pmol/mg protein/10s vs 1756 pmol/mg protein/10s). Na^+ and K^+ gradient independent glutamate transport was not affected by oleic acid.

In difference to glucose and glutamate we observed no effect of OA on the initial transport rates (<15 s) of the citrate and succinate transport system. At 30 s the inhibition of the citrate transport by 500 µM OA was in the range of 7%. Thus these various co-transport systems exhibit differences in their time dependent inhibition caused by oleic acid.

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Proteintransport in Chloroplasts: Import of Overproduced LHCP-Precursor Protein

The precursor protein of the light harvesting chlorophyll a/b binding protein (LHCP) was overproduced in E. coli and purified to greater than 95% purity. This purified precursor protein was used to study import into isolated, intact pea chloroplasts. This system allowed us to elucidate the role of cytosolic factors in the import process. This was not possible until now, since only precursors were used which were synthesized in vitro by wheat germ or reticulocyte lysate system. Results will be presented which demonstrate the function of cytosolic proteins in protein import in chloroplasts.

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Direct Measurement of Single Channel H^+ Currents Through Reconstituted Chloroplast ATP synthase

The purified chloroplast ATP synthase (CF_0-CF_1) was reconstituted into azolectin liposomes from which bilayer membranes on the tip of a glass pipette (»dip stick technique«) and planar bilayer membranes were formed. The chloroplast ATP synthase (CF_0-CF_1) facilitated ion-conductance through the bilayer membranes.
The observed single channel currents were carried by H^+ through the isolated and reconstituted chloroplast ATPase. We demonstrate that it is the intact enzyme complex CF_0-CF_1 and not the membrane sector CF_0 alone that constitutes a voltage-gated, proton selective channel with a high unit conductance of 1-5 pS at pH 5.5-pH 8.0. The open probability P_0 of the CF_0-CF_1 channel increased considerably with increasing membrane voltage (from $P_0 \leq 1\%$ ($V_m \leq 120$ mV) to $P_0 \leq 30\%$ (120 mV $\leq V_m \leq$ 200 mV)). In the presence of ADP (3 μM) and P_i (5 μM), wich specifically bind to CF_1, the open probability decreased and venturicidin (1 μM), a specific inhibitor of H^+ flow through CF_0 in thylakoid membranes, blocked the channel almost completely.
Our results which revealed a high channel unit conductance, and at membrane voltages < 100 mV low open probability with concominant mean open times in the μs range suggest a gated mechanism with channel openings in the μs time scale (< 100 μs) for the energy coupling in the enzyme complex (1).

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Purification and properties of ureidopropionase from calf liver

Ureidopropionase (E.C.3.5.1.6) is the last enzyme in a three-step sequence metabolizing uracil to β-alanine, CO_2 and NH_3 via dihydrouracil and N-carbamoyl-β-alanine. Uracil degradation, the only pathway leading to β-alanine in mammalian tissues, seems to be important in regulating the concentrations of pyrimidine bases required for DNA and RNA synthesis[1]. The inhibition of these catabolic enzymes could support the chemotherapeutic efficiency of clinically applied antineoplastic pyrimidine base analogues, such as 5-fluorouracil[2].
Ureidopropionase was purified from calf liver acetone powder to homogeneity using heat treatment in the presence of 10 mM propionate at 50°C, ammonium sulfate fractionation and chromatography on octyl-Sepharose, DEAE-Sepharose and CM-cellulose with a 46 per cent recovery. The native enzyme has a molecular mass of 231 kDa and consists of 6 subunits with 38.5 kDa each. The pH optimum shows a plateau in the range from pH 5.7 to 7.0. The temperature optimum for the enzymatic hydrolysis of N-carbamoyl-β-alanine at pH 7.0 is 37°C. A shift to higher temperature values is observed in the presence of propionate.
Enzyme activity follows Michaelis-Menten kinetics with a Km value of 0.5 mM for N-carbamoyl-β-alanine which is in contrast to reports on the rat liver enzyme[3]. Alternative substrates are N-carbamoyl-DL-β-aminoisobutyrate, N-carbamoyl-γ-aminobutyrate and N-carbamoylglycine with relative enzymatic activities of 70%, 11% and 4%, respectively. Propionate and 2-methyl butyric acid were found to be competitive inhibitors with Ki values of 35 mM and 13 mM, respectively. In addition, butyric, isobutyric, 2-phenylpropionic and caproic acid inhibit ureidopropionase.

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Cloning, Overexpression, and Partial Sequencing of Genes Coding for Maltose Transport Proteins in Salmonella typhimurium

Salmonella typhimurium, as Escherichia coli can utilize maltose and maltodextrins as sole sources for carbon and energy. These sugars enter the periplasm by passage through porins or maltoporin (LamB) and are taken up into the cytoplasm via a binding-protein-dependent active transport system, most likely on the expense of ATP. The membrane-bound components comprise two hydrophobic integral (MalF, MalG), and a peripheral membrane protein (MalK). The latter has been shown to contain a nucleotide binding site. The genes coding for the protein components of the transport system are organized in two divergently transcribed operons (malB regulon).
In order to characterize the transport system biochemically, all proteins, with the exception of the binding protein MalE have to be overproduced, since they are only present in wild type cells in low amounts. To this end, a clone (pES3) was isolated from a Salmonella gene bank, containing the entire malB region in plasmid vector pBR328. Restriction fragments containing the individual genes malE, malK, malF, and malG, respectively, were isolated and subcloned into different expression vectors, harboring either lambda p_L- and lambda p_R-promoters or a tac/trc promoter. Gene products expressed from these plasmids were identified by in vivo-labeling, in mini-cells, or by SPAGE. The complete nucleotide sequences of malF and malG , and additionally, partial nucleotide sequences of lamB and malM were obtained. Homologies with the corresponding genes of E. coli will be discussed.

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Exchange of cytoplasmic and transmembrane domains of the 46 kDa mannose 6-phosphate receptor (MPR-46) and the lysosomal acid phosphatase (LAP)

The MPR-46 is a transmembrane protein that recycles between trans Golgi network, prelysosomal compartments and the plasmamembrane. While MPR-46 mediates transport of newly synthesized lysosomal enzymes to lysosomes, the receptor is not found within lysosomes. The LAP is transported to the lysosomes as a transmembrane protein - independent of MPR - and processed to a soluble form within the lysosomes.. Mutants of both proteins that lack the cytoplasmic tails are transported to the plasma membrane by bulk flow. This indicates that specific transport signals are harboured within the cytoplasmic tails. Chimeric proteins of MPR-46 and LAP were constructed by in vitro mutagenesis to test this hypothesis. The luminal domain of the MPR-46 was fused to the transmembrane and cytoplasmic domain of the LAP and vice versa. Expression of the chimeric cDNAs in BHK cells resulted in synthesis of functional proteins. The chimeric MPR-46 with the cytoplasmic LAP portion was found to be transported to the lysosomes. Thus, the cytoplasmic tail of the LAP seems to be sufficient to target a non lysosomal membrane protein to the lysosomes. The chimeric LAP with the MPR-46 cytoplasmic tail was transported to the lysosomes at a significantly slower rate than wildtype LAP. This indicates retention of the chimeric LAP within prelysosomal compartments. Furthermore, recycling of the chimeric LAP between the trans Golgi network and plasmamembrane was indicated by resialylation of chimeric LAP, that had been desialylated at the cell surface. Thus, the pathway of the chimeric LAP has features that are characteristic of MPR-46. The signals for retrieval from prelysosomal organelles and recycling to the trans Golgi network are therefore supposed to be located in the cytoplasmic tail of MPR-46.

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Antigenicity of recombinant proteins and synthetic peptides corresponding to HIV sequences

Restriction fragments of pBH10-RIII and synthetic oligonucleotides were cloned in expression vectors in E.coli. Fusion proteins with ß-galactosidase and aa 260-548, aa 548-736, aa 737-863, aa 597-611 of gp160 (env), aa 118-512 of p55 (gag) of HIV-1 and aa 589-603 of gp140 (env) of HIV-2 were tested with 60 positive sera by immunoblotting. Fusion protein aa 589-603 was tested with 12 HIV-2 positive sera.
Fusion protein aa 597-611 was recognized by 100% of sera, 98,3% of the sera reacted with fusion protein aa 548-736. The fusion protein aa 260-548 was detected by 81,6% and fusion protein aa 737-863 by 63,3%. Fusion protein aa 118-512 (gag)

was recognized by 43,3% of sera corresponding to different CDC groups.
The fusion protein aa 589-603 was recognized by 100% of HIV-2 positive sera.
Synthetic peptides were prepared via the Fmoc-strategy and purified by reversed phase chromatography.
Peptides were selected corresponding to HIV-1 gag aa 105-115, aa 129-135, aa 234-243 and HIV-1 env aa 504-518, aa 507-518, aa 512-518 and HIV-2 env aa 589-603 and aa 597-603.
The same sera were applied to an ELISA-test with the synthetic peptides. The peptide gag aa 105-115 was detected by 23,3%, aa 129-135 by 41,7%, and aa 234-243 by 3,3% of sera. Peptide env aa 504-518 was recognized by 63,3%, aa 507-518 by 73,3%, aa 512-518 by 61,7%, and aa 597-611 by 100% of sera. Peptides env aa 589-603, and aa 597-603 from HIV-2 were both recognized by 91,7% of sera.
Antigenicity of recombinant proteins and synthetic peptides was demonstrated to be comparable.

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ion. There is evidence from Gua-HCl unfolding curves that a breakdown of the structure is followed by dissociation into monomers.

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Mutations and Protein Stability: Thermodynamic and Structural Studies on ROP Protein

ColE1 rop is a small protein of 63 amino acid which is involved in the control of the frequencies of replication initiation events of ColE1 and related plasmids[1,2]. In solution the molecule occurs as a dimer. Each monomer consists of two antiparallel alpha-helices, which are connected by a hairpin bend of about four amino acids[3]. The strictly helical parts of the dimer can be approximated by a four stranded rope of left-handed twist and square cross section.
Our studies concentrated on the significance of the bend region for structure and stability of the rop molecule. Oligonucleotide-directed mutagenesis has provided a large number of amino acid alterations both on the surface[4] as well as in the hydrophobic core of the protein. The mutants we were concerned with in this study are: RM6, having a deletion of amino acids 30-34; RM7, having two Ala inserted in the bend region, and Ala31Pro, having Ala 31 mutated to Pro.
Stability studies were based on differential scanning calorimetry (DSC) measurements, and temperature- or guanidinium-HCl-induced denaturation curves, that were monitored by following the change of the circular-dichroism signal in the far UV-region.
All mutations decreased the stability of the rop dimer. Native rop requires a Gibbs free energy input of 75 kJ/mol for unfolding, the RM7 insertion mutant exhibits a ΔG of 65 kJ/mol. Dramatic destabilization results from both deletion of the amino acids in the bend region (RM6) and introduction of a proline in position 31. The corresponding stabilities are only 35 kJ/mol and 20 kJ/mol at 25°C, respectively.
Thus interactions in the four helix bundle are least perturbed by the insertion of 2 Ala in the bend, most by replacement of Ala 31 by Pro. Ala31Pro does nor appear to unfold in a two-state transit-

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Heterogeneity of the P-Glycoprotein Gene Region in Three Independently Selected Multidrug Resistant Human Leukemia Cell Lines

Multidrug resistant (MDR) human lymphoblastoid CCRF-CEM cell lines were selected in vitro with either actinomycin D, vincristine or adriamycin, respectively. In any case amplification and high expression of a P-glycoprotein gene (mdr1) was detected. The genomic analysis of the mdr gene region in the sensitive parental and the resistant CCRF sublines revealed restriction fragment lengths polymorphisms (RFLPs) using either conventional agarose gel electrophoresis or field alternation gel electrophoresis for separation of very large DNA molecules (1). The cytogenetic characterisation of the various sublines revealed marked differences as well: The actinomycin D selected subline exhibited no chromosomal peculiarities in comparison with the sensitive CCRF cell line. Using a mdr1 gene probe, the P-glycoprotein gene could be localized at chromosome 7q21.1-22.1 by *in situ* hybridization experiments. The vincristine selected subline showed a trisomy 7 with an abnormally banding region on the long arm of one of the chromosomes 7. In case of the adriamycin selected subline a pericentric inversion on chromosome 7 was detected with a break at 7q22 which is a common fragile site in humans. The three MDR sublines showed distinct variations of the cross resistance patterns. Because in such cases sequence alterations of the mdr1 cDNA around the base pair 555 were recently reported by others (2), we asked whether the same might be true here. Employing the PCR (polymerase chain reaction) technique we cloned respective fragments out of each subline. However, the

sequencing of 15 recombinants did not show any significant sequence heterogeneity between the DNA fragments from the different sources. With one exception the sequences obtained were all identical with one of the sequences published (2).

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Verwendung chromosomaler lacZ-Fusionen bei Klebsiella pneumoniae zur Genkartierung und für Regulationsstudien beim sor-Operon

Das PEP-abhängige bakterielle Phosphotransferase System (PTS) katalysiert die Aufnahme diverser Kohlenhydrate und leitet damit auch deren Verstoffwechselung ein [1]. Zu diesen PTS-abhängigen Zuckern gehört die Ketose L-Sorbose, die von Klebsiella pneumoniae, jedoch nicht von den meisten E.coli-Stämmen (z.B.K-12), verstoffwechselt werden kann.
Mit Hilfe von λplacMu-Phagen haben wir auch bei λ-sensitiven Klebsiella-Stämmen chromosomale lacZ-Fusionen, unter anderem im sor-Operon, isoliert. Diese dienen zum einen als Basis für die Isolierung stabiler Hfr-Stämme für Kartierungen bei Klebsiella [2], zum anderen für Regulationsuntersuchungen an den sor-Genen.
Das sor-Operon ist ebenfalls kloniert worden und es wurden folgende Strukturgene und deren Genprodukte nachgewiesen: sorA kodiert für ein membrangebundenes EII^{sor}, sorF und sorB sind die Strukturgene für zwei lösliche $EIII^{sor}$-Proteine, sorE ist das Strukturgen einer Sorbose 1-Phosphatreduktase, sorD kodiert eine Glucitol 6-Phosphatdehydrogenase und sorC das Regulationsprotein (Wöhrl und Lengeler, in Vorbereitung). Die Strukturgene sind als Operon anscheinend hinter einem gemeinsamen Promotor angeordnet. Anhand von Komplementationstests wird gezeigt, daß SorC sowohl Aktivator als auch Repressorfunktion besitzt, was, in Verbindung mit nur einem Promotor für das gesamte Operon, auf eine sehr komplexe Regulation in der Promotorregion schließen läßt.

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Calcium Influx and Efflux Systems in the Plasmamembrane of a Higher Plant

As in other eucaryotic cells, calcium has multiple regulatory functions in plant cells. However, calcium transport systems have not been isolated to date from plant sources. We have identified and characterized a Ca^{2+}-ATPase and a Ca^{2+}-channel in highly pure plasmalemma vesicles from leaves of the monocotyledonous plant, *Commelina communis* L. The Ca^{2+}-channel is open when the membrane is depolarized, Ca^{2+}-inactivated, blocked by La^{3+} and directs Ca^{2+} entry into the cell. The Ca^{2+}-ATPase has a low K_m for Ca^{2+} (4.4 μM) and exports Ca^{2+} from the cell. The enzyme was solubilized, reconstituted in highly functional form and is currently being purified. The role of these Ca^{2+} transporters in the regulation of ion transport and cell metabolism will be discussed.

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Temporary Activation of the Nuclear Double-stranded RNA-dependent (2'-5')A Synthetase/RNase L System after Infection of H9 Cells with HIV-1

Human T cells (H9), infected with the HTLV-IIIB strain of the human immunodeficiency virus (HIV-1), have been used to study the alteration of 2',5'-oligoadenylate ((2'-5')A) metabolism in relation to virus production. The synthesis of (2'-5')A was determined to proceed in close association with the nuclear matrix. After HIV infection the (2'-5')A synthetase activity increased from 1.1-1.5 pmol of (2'-5')A synthesized/100 μg of nuclear matrix protein (during a 3-h in vitro incubation period) to 8.2 pmol at day 3 after infection. Then the activity dropped to the initial values. In noninfected H9 cells the (2'-5')A synthetase activity remained unchanged. Simultaneously with the decrease in the (2'-5')A level the cells started to release HIV. At the time of maximum synthetase levels the (2'-5')A-activated endoribonuclease (RNase L) activity strongly increased. Only one protein could be selectively cross-linked to a (2'-5')A derivative in the nuclear matrix from H9 cells; this protein is assumed to be RNAse L. Experimental evidence is provided revealing that RNase L degrades HIV transcripts. A correlation could be established between high levels of (2'-5')A and RNase L and a failure of the cells to release HIV. 3'-Azido-3'-deoxythymidine was shown to cause an extension of the time period during which an RNase L-mediated degradation of viral transcripts occurred. The possibility of a novel molecular pharmacologic approach on the level of (2'-5')A metabolism is discussed.

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The Semisynthesis and Characterization of $Pro^{15}(P_1)$-Aprotinin

Most naturally occurring serine proteinase inhibitors associate with their target enzymes, in an analogous way to the association of a substrate. Thereby, they show optimal binding properties and form extremely stable complexes. Starting with aprotinin, the bovine Kunitz inhibitor, we succeeded, by varying the P_1 amino acid residue, in semisynthesizing a whole series of homologues, whose specific inhibitory properties are markedly different[1,2]. All the built in amino acids, coded and uncoded, have hitherto had a primary α-amino group. The incorporation of proline was of especial interest, because its secondary α-amino group and its relatively rigid structure are exceptional. It occurs as the P_1 residue in several Kazal inhibitor domains. An inhibitory activity of these proteins could hitherto not be demonstrated[3].

Our general technique for the 'chemical mutation' of the P_1 residue of aprotinin[1] was successfully used for the substitution of lysine by proline. The reaction times for both of the carbodiimide coupling steps had, however, to be extended. Pro^{15}-aprotinin was characterized using cation exchange HPLC, amino acid analyis, and automated sequencing. Neither bovine trypsin (EC 3.4.21.4), bovine chymotrypsin (EC 3.4.21.1) nor kallikrein of porcine pancreas (EC 3.4. 21.35) were inhibited. Significant inhibition could only be shown of elastase from human leukocytes (EC 3.4.21.37). The dissociation constant, of about 10^{-5} M, for the corresponding complex is, however, very high and is five orders of magnitude above that of the elastase/Val^{15}-aprotinin complex.

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Polyol Uptake in Rat Glial Primary Cultures

Astroglia-rich rat primary cultures can be cultivated in a glucose-free medium in the presence of 25 mM sorbitol. Sorbitol is taken up into the glial cells by a diffusion process, not involving a carrier and probably not through the lipid bilayer, but through a proteinaceous pore-like structure[1]. On the other hand accumulation of endogenously produced sorbitol may be a critical factor in the etiology of diabetic complications in cells of the peripheral nervous system[2].

In order to gain more information on the permeability of polyols in glial cells a systematic uptake study with polyols with varying chain lengths (mannitol, xylitol, erythritol, glycerol) was performed. Time and concentration dependence of uptake was investigated for each compound. All polyols enter the glial cells by a simple diffusion mechanism with only slight differences in the initial rates of uptake. Phloretin inhibits polyol uptake to various degrees; glycerol shows exceptionel behaviour in that uptake into glial cells is enhanced in the presence of phloretin.

In contrast, rate of uptake of polyols in C-6 glioma cells is an order of magnitude lower as compared with the glial primary cultures. Therefore the magnitude of uptake of polyols seems to be a particular property of cells, and only complete lack of permeability of polyols may render certain cell types susceptible to pathological changes in persistent diabetes.

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The influence of lectins on the synthesis of different glycosaminoglycan fractions of human fibroblasts in tissue culture

Glycosaminoglycans (GAGs) are major constituents of the extracellular matrix and are secreted by fibroblasts. Previous studies have shown the GAG synthesis can be modulated by the addition of lectins. Now we wanted to investigate the influ-ence of the lectins on different GAG species, namely on hyaluronic acid (HA) and sulphated GAGs (sGAGs). The incorporation of ^{14}C-glucosamine was measured under the influence of the following lectins: Concanavalin A (Con A), wheatgerm agglutinin (WGA) and soybean agglutinin (SBA). After pulsing the cells with the radioactive material the GAGs were separated by ion exchange chromatography. The GAG synthesis was stimulated by Con A, WGA and SBA (at 10 ug lectin/ml medium). Most of the radioactivity was incorporated in the glycopeptide fraction (90%). The HA fraction accounted for about 5% and the sGAGs for about 2%. All the lectins used stimulated these three fractions in the same fashion. The similar results after stimulation of GAG synthesis by

three lectins with different sugar specificities can be explained: 1)the fibroblast in culture is not able to alter the ratio of different GAGs or 2)the stimulation by the lectins leads to a general increase of the metabolic activities. We favour the first explanation since we found in earlier studies different reactions of the fibroblasts's DNA- and protein-biosynthesis upon stimulation with various lectins indicating the stimulation of different receptors.

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Investigation of a Phenylalanine Transport System in Isolated Barley Vayuoles

Intact vacuoles have been prepared from barley mesophyll protoplasts according to the method of Kaiser et al. (1). Phenylalanine uptake into the vacuoles can be driven by both ATPase (EC 3.6.1.3) and PPase (EC 3.6.1.1) (2,3). ATPase and ATPase driven amino acid transport is inhibited by nitrate (90 % inhibition by 40 mM nitrate) whereas PPase and PPase driven transport are not affected. Azide, vanadate and oligomycin have no effect. Destruction of the electrochemical gradient by addition of nigericin, valinomycin or gramicidin results in complete inhibition of ATP or PP_i dependent phenylalanine transport but 2-fold stimulation of ATPase and PPase activities. pH-optima for ATPase and PPase driven transport are 7.3 and 7.8, respectively (4). The carrier sytem shows high stereospecificity for hydrophobic L-amino acids. L-Ala, L-Val, D-Phe and D-Leu have no effect on phenylalanine transport whereas L-Ile, L-Leu and L-Trp inhibit strongly (44-75 %). L-stereospecificity can be overcome by high hydrophobicity as presence of D-Trp results in over 50 % inhibition. Phenylalanine analogues (p-F-L-Phe, p-NH_2-L-Phe and p-NO_2-L-Phe) and L-Tyr all compete effectively with phenylalanine transport (30-50 % inhibition). Presence of the α-amino but not the carboxyl group is essential for recognition. Phenylpyruvate and indol acetic acid are ineffective whereas the inhibitory effect of hordenine, tryptamine and gramine is in the range of D-Trp and L-Trp, respectively. These results show that a highly stereospecific transport system for aromatic L-amino acids exists in vacuolar membranes. Further characterization is under investigation.

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Interaction of Small Dermatan Sulfate Proteoglycan II with Fibronectin and Its Influence on Fibroblast Adhesion

Cell adhesion is a complex process involving several extracellular matrix components, the integrins, membrane-intercalated heparan sulfate proteoglycans, and the cytoskeleton. The hypothesis has been put forward that heparan sulfate proteoglycans may link intracellular cytosceletal components with the matrix protein fibronectin. We have shown previously that the core protein of small dermatan sulfate proteoglycan II (DS-PG II) from human fibroblasts interacts with fibronectin (1). It binds to the N-terminal as well as to the heparin-binding domain near the C-terminal end. The recently discovered interaction with a cell-binding fibronectin fragment of 105 kD interferes with the binding of monoclonal antibodies to DS-PG II core protein. Micromolar concentration of the pentapeptide NKISK the sequence of which is found in several repeats of the core protein, inhibit in part the interaction of core protein and intact fibronectin in an ELISA test system.

To study the biological significance of the DS-PG II fibronectin interactions, we investigated the effect of intact proteoglycan and of its core protein on the adhesion of fibroblasts to various substrata. DS-PG II and its glycosaminoglycan-free core protein inhibited the adhesion-promoting effect of intact fibronectin and of its cell-binding fragments. Adhesion of fibroblasts to heparin-binding domains was quantitatively similar to adhesion on plastic surfaces, but DS-PG II and core protein were inhibitory in this case, too.

Cell adhesion was also reduced on DS-PG II- or core protein-coated surfaces. These results suggest that DS-PG II which is ubiquitously distributed, could play a regulatory role in cell adhesion.

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Structural organization of gangliosides in bilayer membranes

Gangliosides are particulary abundant in cerebral grey matter but are also present in extraneuronal tissue. Their function and interaction with other lipid components and membrane proteins is far from being understood. It has been supposed that gangliosides serve as Ca^{2+}-storage and regulate nerve cell membrane permeability. Ganglioside function is probably dependent on the chemical structure of the gangliosides and also on the conformational state of their headgroup. This is especially interesting with respect to their function as a receptor for different toxins.

Pyrene labeled gangliosides were used to study their distribution in phosphatidylcholine bilayer membranes by the excimer formation technique. Labeled disialogangliosides PyG_{D1a} and PyG_{D1b} exemplify a system with nearly ideal mixing even in the precence of Ca^{2+}. Monosialogangliosides exhibit a preference for fluid bilayer regions. We observe a passive exclusion of G_{M3}, G_{M2} and G_{M1} from phosphatidylcholine domains that are rigidified by the Ca-ions. Our experiments exclude a specific ganglioside-Ca^{2+}-interaction. In phosphatidylethanolamine bilayer membranes the lipid phase transition temperature of fully hydrated membranes was reduced by G_{D1a} due to a disturbance of the hydrogen bonds between PE-molecules. This could not be observed with G_{M1} or G_{T1b}. From the spectroscopic determination of the hydration-dehydration process of PE membranes we were able to get information on the ganglioside headgroup orientation.

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Preparation of Isoguanosine (Crotonoside) and Adenosine N-oxides as Nucleoside HPLC-Standards and Possible Constituents of a Monocytic Metallo-Ribonucleo-Polypeptide (Cu-RNP) Angio-Morphogen

We elaborated structural and functional properties of a monocytic organogenetic tissue hormone (monocyto-angiotropin) purified (>100'000-fold) to homogeneity from supernatant solutions of serum-free cultures of isolated, lectin-activated porcine peripheral monocytes (macrophages). Its angio-morphogenetic (blood vessel-forming) bioactivity is expressed in vivo and vitro on endothelial cells as molecular property of (femto mols of) an extracellular Cu-RNP (1-4). Its RNA portion (75 bases) was found peculiar since containing "unusual" nucleobases (151 Da) isolated by HPLC after precolumn hydrolysis and characterized by different (chromatographic and spectrometric) methods (1,5).

To investigate whether nucleobase isomer (other than "usual" guanine) of 151Da (isoguanine, adenine N(1,3,7)-oxides, 6-hydroxylaminopurine, 8-hydroxyadenine) represents the modified constituent, some of the isomeric nucleobase and, especially, correspondent nucleoside standards had to be prepared, since unavailable in HPLC-pure form. Hence, several earlier established methods were tried by either (a) pH-dependent and/or metal ion-catalyzed random oxidation of adenosine by OH-radicals (6), (b) synthesis of crotonoside from 2,6-diamino-9-ß-D-ribofuranosylpurine (7), or (c) photochemical disproportionation of adenosine N1-oxide to isoguanosine/ adenosine (8). The earlier purification procedures lacking HPLC for the numerous mixed compounds formed by either method were accomplished and/or replaced by reverse phase HPLC combined with spectrometry. Identity of a prepared isoguanosine HPLC-standard to the splitted modified nucleobase/nucleoside component was found in chromatographic and spectrometric terms. Nevertheless, a possibility remains that a tautomer of adenosine N1-oxide might be the true constituent in the intact, bioactive Cu-RNP: Upon hydrolysis, it might disproportionate to yield the finally detectable isoguanine/isoguanosine. It is suggested that the Cu-RNP monokine structure paracrinely bioactive on endothelial cells, results from postsynthetic or postsecretory modification in an autocrine fashion by OH-radicals (as another macrophage product) within the subtle interplay of inflammation and regenerative tissue morphogenesis (wound healing).

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Fragmentation of Small Dermatan Sulfate Proteoglycan Core Proteins in Human Cartilage Tissue

In addition to large aggregating proteoglycans cartilage contains two types of small dermatan sulfate proteoglycans, DS-PG I and DS-PG II, which carry only 1 (DS-PG II) and 2 (DS-PG I) glycosaminoglycan chains[1], respectively. Because of the ability of small proteoglycans to interact with interstitial collagens[2] via their core proteins and because of the

contribution of the glycosaminoglycan moiety to the organization of the collagen network, DS-PG I and II could play a role in providing the tensile strength of cartilage.

We have analyzed macroscopically normal cartilage from human femoral condyles as a basis to study possible changes during the development of osteoarthritis. Tissue extracts were chromatographed on DEAE-Trisacryl and subjected to SDS/polyacrylamide gel electrophoresis and Western blotting before and after enzymatic removal of the glycosaminoglycan chains. The blots were stained with monospecific polyclonal antibodies against the core proteins of DS-PG I and II, respectively. Small amounts of glycosaminoglycan-free DS-PG I core protein of 37 kDa were detected in the tissue which could have been arisen by a single proteolytic clip near the N-terminus. The core protein of glycosaminoglycan chain-bearing PG I was intact. In contrast, at least 25% of DS-PG II core protein was fragmented. Glycosaminoglycan-free core protein of 44 and 48 kDa and chain-bearing fragments of 38, 35, 30, 23, and 17 kDa were regularly found. These results demonstrate that even normal cartilage contains partially degraded small proteoglycan core proteins.

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Active Lactose Permease Produced by Regulated Co-expression of Two *lacY* DNA Segments Encoding Complementary Amino Acid Sequences

Lactose permease, the product of the *lacY* gene of *E. coli*, is one of the best analyzed transport proteins. We have chosen it as a model to define more precisely regions important for membrane insertion of this polytopic membrane protein. Guided by published models for the structure of lactose permease we have constructed and analyzed mutant *lacY* proteins. The residual proteins encoded by several deletion mutations to be shown represent permease segments predicted to form approximately complete α-helices. Expression of the mutant genes was analyzed in *E. coli* minicells and in growing cells by gelelectrophoretic and immunological methods [1,2].

Mutant proteins were also characterized with respect to membrane association and transport activity. Of particular interest is plasmid pAY which contains two *lacY* DNA segments encoding separate complementary lactose permease polypeptides. Both carry identical *lacOP* regions governing their transcription.

Co-expression of these *lacY* sequences in transport-negative cells resulted in restoration of active transport measured as accumulation of ^{14}C-lactose. The larger polypeptide YΔ 4-69 (amino acid residues 4-69 of the lactose permease sequence deleted) is complemented by the N-terminal polypeptide comprising residues 1-71, the region predicted to form the first two α-helices of the intact protein. This smaller polypeptide was also required for the larger polypeptide to exhibit specific substrate binding and reactivity with an IgG preparation directed against the C-terminus of *lac* permease.

These results together with earlier data[1-3] suggest that an N-terminal segment of lactose permease exhibits a certain degree of structural and functional autonomy.

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EII specific for the glucose PTS of Staphylococcus carnosus

The phosphotransferase system in Gram positive bacteria consists of the constitutive enzymes EI and HPr and the inducible, sugar-specific enzymes EIII and EII [1].

In Gram negative bacteria there also exists PTS where a hydrophilic domain of EII on the cytoplasmic side of the membrane overtakes the function of a EIII, so that you can speak about a EII/EIII-fusion protein [2].

The glucose-specific PTS of Staphylococcus carnosus is induced after growth on glucose-, lactose- or mannitol-containing medium.

Phosphorylation activities of the membranes are tested by addition of EI, HPr and a ^{3}H-2-deoxyglucose-assay.

Comparison of the membrane phosphorylation activity from bacteria, which have grown on glycerol- or on glucose-medium, results in a 70-fold induction.

Biochemical investigations and phosphorylation of the enzymes with ^{32}P-PEP showed that the glucose-specific PTS of Staphylococcus carnosus consists of the enzymes EI, HPr and EII^{glc} but no $EIII^{glc}$ could be detected.

EII^{glc} is a 70kd protein. The molecular size of the enzyme indicates that a hydrophilic domain assumes the function of EIII.

Sato et al. [3] recently had sequenced the sucrose-specific EII of Streptococcus mutans. Its molecular weight is 69983. A soluble , sugar-specific EIII could not be detected.

So besides the sucrose-specific PTS in Streptococcus mutans a second system with a EII/EIII-fusion protein is found in Gram positive bacteria.

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Studies on the Growth Rate Dependence and Stringent Sensitivity of Transcription from Ribosomal RNA Promoter Variants

The transcription initiation of many *E. coli* promoters which direct the synthesis of stable RNA molecules (rRNA and tRNA) is growth rate regulated and sensitive to amino acid starvation. It is assumed that a GC-rich 'discriminator' motif proximal to the -10 promoter region plays an important role in determining the regulative features of these promoters[1].

Using site directed mutagenesis we have altered both the *rrnB* P2 and the synthetic *tac* promoter[2] to the consensus GCGC discriminator motif. The modified promoters were placed upstream of the structural gene encoding for the chloramphenicol acetyltransferase (CAT). The response of the modified promoters to amino acid starvation, changes in the growth rate, or differences in the basal level of guanosine tetraphosphate (ppGpp) were determined *in vivo*. The results clearly show, that the discriminator motif is sufficient to convert the ribosomal RNA promoter P2 to a stringent- as well as growth rate regulated promoter. By contrast, the same discriminator sequence linked to the synthetic *tac* promoter does not convert this promoter to either stringency or growth rate regulation. Finally, the results presented in this study reinforce the view that stringent and growth rate regulation utilize the same mechanism, with ppGpp being the common mediator.

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Partial Purification and Functional Reconstitution of a Voltage-dependent Potassium Channel from *Vicia faba* L.

Plasma membranes prepared from leaves of *Vicia faba* L. by aqueous two-phase partitioning exhibited Rb^+-uptake activity measured as $^{86}Rb^+$ flux into vesicles in the presence of symmetrical K^+ = 50 mM. This process was sensitive to tetraethylammonium ions (TEA, IC_{50} = 5 mM) and $AlCl_3$ (IC_{50} = 0.5 mM). Ion selectivity was monitored in vesicles loaded with $^{86}Rb^+$ in presence of Rb^+, K^+, Na^+, Li^+ and choline$^+$. The $^{86}Rb^+$-transport system was solubilized with the detergent nonanoyl-N-methylglucamide (Mega 9, 60 mM, detergent to protein ratio = 20) and functionally reconstituted into liposomes by detergent dialysis technique. The progress of purification of the system was checked using the acquisition of TEA-sensitive $^{86}Rb^+$-transport activity of fractions reconstituted into liposomes as a functional assay. The transport activity copurified with a protein band with an apparent molceular mass of 72 ± 2 kDa as determined by sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE). In some preparations additional protein bands were observed (between 43 and 68 kDa). Channel fluctuations

were observed after incorporation of the partially purified protein into planar lipid bilayers. A single conductance of 40 pS was estimated, indicating that the purified $^{86}Rb^+$-transport activity represents a cation and most likely, a potassium channel.

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A Model to Study Transendothelial Transport (TET)

The endothelial cell monolayer builds up a distinct barrier between blood and tissue. The extracellular diffusion of molecules is restricted by gaps between the cells. The ability of selective substrate uptake and release by the cells characterizes the intracellular pathway (1). To study the mechanisms regulating TET we used an endothelial cell monolayer which seperated the incubation beaker into two compartments. Bovine aortic endothelial cells were seeded on polycarbonate filters (pore size: 0.4µm). Studies of diffusion kinetics were carried out with ^{14}C-sucrose, inulin and FITC dextranes of different molecular weights. Sucrose as a diffusion marker (2) passed the monolayer to 70% in 1h. Its diffusion was not dependent on concentration and hence not saturable. The velocity (pmoles/cm^2/h) of diffusion remained constant over the period of incubation (up to 3h) and it is increasing linearly with the concentration of tracer. FITC dextranes cannot penetrate the cell membrane therefore we took them as markers for extracellular diffusion. TET of FITC dextranes was also not saturable and was dependent on the molecular weight of the substrates. FITC dextrane (MW 70000d) passed the monolayer up to 4% in 1h whereas FITC dextrane (MW 150000d) was nearly not able to pass the monolayer. Diffusion is temperature dependent. Calculating Arrhenius equation 24.5 KJ/mol for sucrose and 22.7 KJ/mol for FITC dextrane (MW 70000d) were determined. Additionally, transmission EM made sure that junctions between the cells existed. Therefore we assume that our model is useful for further investigations as for example TET of hormones and modification of TET by hormones.

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Membrane Energization alters Structure and Function of ATP-Synthase from Micrococcus luteus

Protoplast membrane vesicles (PMV) from the aerobic bacterium Micrococcus luteus contain an intact respiratory chain additionally to ATP-Synthase (EC.3.6.1.34). Thus PMV are suitable for the investigation of proposed membrane energization dependent structural changes during the catalytic reaction of ATP-synthase and its regulation(1,2).

Inside out orientated PMV were prepared from freshly grown bacteria by sonication. The membranes were energized by oxidation of various concentrations of NADH. The PMV were capable of NADH driven ATP-synthesis, which was sensitive to the ATP-synthase specific inhibitors dicyclohexylcarbodiimide and Oligomycin. The energization of the membranes resulted in an activation of the MgATP hydrolysis activity. Only at high NADH concentrations the expected proton potential dependent inhibition of ATP-synthase was observed. The activation effect was abolished by the uncoupler FCCP, which suggests an interconversion of a less active resting form into a highly active modification of ATP-synthase by the electrochemical proton potential difference of the membranes. The effect of the membrane energization on the structure of the F_0moiety of ATP-synthase was estimated by inhibition of the enzyme by Oligomycine and dicyclohexylcarbodiimide. Energized ATP-synthase reacted more strongly with both inhibitors as compared to the non-energized enzyme. The enhancement of the inhibitor reactions by the membrane energization was sensitive to the uncoupler FCCP.

Thus it was concluded that structure and function of the F_1- as well as the F_0moiety of ATP-synthase is altered by membrane energization.

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