Miscellaneous

M01

SUBCELLULAR DISTRIBUTION OF GPI-PLD

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Glycosylphosphatidylinositol-specific phospholipase D (GPI-PLD) is present in high amounts in mammalian serum, as well as in milk and cerebrospinal fluid, from where it may be taken up by cells and play a role in the intracellular degradation of GPI structures. In order to localize possible sites of action of GPI-PLD, we studied its subcellular distribution in rat liver. Purified GPI-PLD shows maximal activity at the critical micellar concentration of Triton X-100, however, this maximum is affected by the presence of lipids. In order to compare the GPI-PLD activities between the various subcellular fractions, optimal Triton X-100 concentrations were determined for all fractions. Our results show that the majority of cell-associated GPI-PLD activity was found in a lysosomal fraction. Since GPI-PLD was described to be relatively stable towards proteases, lysosomes may represent a possible site of action of GPI-PLD *in vivo*.

M02

DIMERISATION OF LACTASE-PHLORIZIN HYDROLASE OCCURS IN THE ENDOPLASMIC RETICULUM (ER), INVOLVES THE MEMBRANE-SPANNING DOMAIN AND IS REQUIRED FOR AN EFFICIENT TRANSPORT OF THE ENZYME TO THE CELL SURFACE

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Analysis of the quaternary structure of human intestinal lactase-phlorizin hydrolase (LPH) by chemical cross-linking and sucrose-gradient centrifugation reveals that the brush border form of LPH (LPHß; 160-kDa) is a homodimeric molecule. Dimerisation ensues in the ER when LPH is still exclusively found as an uncleaved mannose-rich precursor (pro-LPH$_h$; 215-kDa). Dimerisation is essential for the transport competence of pro-LPH and is strongly associated with the presence of an intact transmembrane domain. Mutant pro-LPH-*mact* lacking the complete transmembrane domain persists as a monomeric, mannose-rich and transport-incompetent molecule that is not secreted into the exterior milieu, accumulates most likely in the ER and is ultimately degraded. Further, deletion of the cytoplasmic tail in the pro-LPH-*ct* mutant leads to marked reduction in the proportion of dimeric as well as complex glycosylated pro-LPH-*ct*. Finally, dimerisation is linked to the acquisition of LPH to its biological function, since only dimers of wild type pro-LPH or pro-LPH-*ct* are enzymatically active, while their monomeric counterparts as well as pro-LPH-*mact* are not.

M03

INTUSSUSCEPTIVE MICROVASCULAR GROWTH IN THE CHOROID OF THE EYE: 3D RECONSTRUCTION OF A TISSUE PILLAR

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In addition to the mechanism of capillary sprouting the process of intussusceptive microvascular growth (IMG) was recently shown to play a role in the enlargement of the capillary bed. IMG involves the formation of a new tissue bridge across an existing capillary lumen. This bridge exhibits a changing morphology, but it is always wrapped by endothelial cells and contains connective tissue at the end of the process. In a previous study we have demonstrated by scanning electron microscopy the presence of tiny transcapillary holes in Mercox casts of the choroid microvasculature. Such holes, with a typical diameter of 1 to 2.5µm, have been shown to represent the landmarks of IMG in the lung. In this study we prove by investigating serial electron microscopic sections, that the holes in the choroid casts also correspond to transcapillary tissue pillars. In order to illustrate the three dimensional aspects of these pillars we reconstructed the serial sections by means of a computer generated surface rendering tool.
Our findings provide further data for the concept that IMG could be an ubiquitous mechanism for the enlargement of the capillary bed.

M04

Proinsulin conversion by endoproteases PC2 & PC3

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Proinsulin (PI) conversion to insulin occurs in granules of pancreatic B-cells. These granules contain two endoproteases, PC2 and PC3. It has been suggested that both enzymes are needed for conversion. We have compared conversion of human PI in GH3 cells (only PC2) with that in AtT20 cells (only PC3). Human PI was expressed using a recombinant adenovirus and cells were pulse-chased. After 60' chase >50% labeled PI was converted in AtT20 cells vs <10% in GH3 cells. Des-31.32 split PI (in which cleavage has only occurred between B-chain and C-peptide) was the major conversion intermediate in both cells and not des-64,65-split proinsulin (the intermediate in which cleavage has only occurred between C-peptide and A-chain), an intermediate previously thought to be generated by PC2 and thus expected in GH3 cells. We conclude that the endoprotease PC2 can convert proinsulin to insulin, though with a much lower efficiency than the endoprotease PC3.

M05

FORMATION OF FILAMENTOUS MICROSTRUCTURES FROM LIPID/CHOLESTEROL VESICLES BY CHAPS

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Phospholipid/cholesterol vesicles were treated with CHAPS at different concentrations in order to study solubilization. At high detergent concentrations mixed micelles were formed with all lipid mixtures. In the presence of negatively charged phospholipids (≥ 70%) the formation of filamentous and helical microstructures was observed, between the vesicular and the micellar regime. Screening of charge by high ionic strength prevented filament formation. The microstructures were also observed, when concentrated micellar phosphatidylcholine/cholesterol mixtures were diluted to form a cholesterol supersaturated state. The microstructures were analyzed by darkfield microscopy, electron microscopy and X-ray diffraction. The fibers had a length of several 100 µm and a diameter of about 90 nm. The helical ribbons had diameters up to 20 µm and occasionally transformed into tubes, which are supposed to be early stages of cholesterol monohydrate crystal formation. The X-ray diffraction pattern (powder) of the fibers was different from both the cholesterol monohydrate and the anhydrous cholesterol crystal pattern. The results are compared to cholesterol precipitation in bile.

M06

CAPILLARY GROWTH IN THE CHICKEN CHORIO-ALLANTOIC MEMBRANE

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In the present study, we examined the capillary growth in the chicken chorio-allantioc membrane (CAM) quantitatively. So far, it is still unknown whether and to what extent the capillary growth is established via capillary sprouting or by the insertion of transcapillary tissue pillars - defined as meshes with a diameter smaller than 2.5µm. The latter process to form new intercapillary meshes is known as intussusceptive microvascular growth (IMG).
The capillary network of the CAM has been labelled by infusion of 8 nm gold particles into 24 chicken embryos aged between 7 and 14 days post incubation. After fixation, the CAMs were removed, silver-enhanced and counterstained with nuclear fast red. The vascular network could then be identified unambiguously and quantitatively analyzed in the light microscope.
In a first stage (day 7) the CAM showed mainly large intercapillary meshes. Between days 8-12 (intermediate stage), the CAM went through a period where numerous small meshes and pillars could be observed. In the last stage (days 13-14), larger intercapillary meshes were again dominant, whereas the number of pillars decreased. From these data we conclude, that during the intermediate stage of development, IMG appears to be a predominant mechanism for capillary growth in the CAM.

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M07

Free-living walking behavior assessed by accelerometry in lean and obese women.

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We used a tri-dimensional accelerometer to estimate the duration, speed and heart rate (HR) of spontaneous walking in a group of obese (wt=101.2 ± 5.1 kg) and lean women (wt = 55 ± 1.7 kg). Each subject walked on a treadmill at 13 combinations of speed/slope and was measured during one afternoon under free-living conditions. The obese selected a significantly lower velocity (3.4 ± 0.1 km/h) than the lean women (4.1 ± 0.2 km/h; $p<0.05$). Obesity resulted in a significantly higher HR at all speeds ($p=0.001$). In both groups an increase in velocity of 1 km/h resulted in an increase in HR of 10 beats/min., whereas the 46 kg group difference in body weight was also associated with a rise in HR of 10 beats/min. Therefore the reduced speed observed in the obese allowed the maintenance of a HR similar to the lean subjects indicating that the obese reduce their intensity of exertion levels to reach that of their lean counterparts.

M08

ENDURANCE CAPACITY AFTER SIMULATED ALTITUDE TRAINING (4000 M); C. Zuleger[1], J. Geiser[1], M. Vogt[2], H. Claassen[2], A. Puntschart[2], R. Billeter[2], H. Hoppeler[2] ([1]Institute of Physiology, Uni of Fribourg, CH-1700 Fribourg, Switzerland; [2]Department of Anatomy, Uni of Bern, CH-3012 Bern, Switzerland)

Effects of ergometer endurance training in hypoxia (H) without concomitant acclimatization to altitude were studied in 35 non-endurance trained male subjects. Half of the subjects trained in normobaric H, the other half served as normoxic control (N). In each group the subjects were randomly assigned to either a high intensity training (HI, 4-6 mmol lactate or 86% HRmax) or a low intensity training (LI, 2-3 mmol lactate or 78% HRmax). During the six weeks of 30 min daily training, power was gradually increased as to maintain the relative effort constant. Results of tests in normoxia: Max. maintained power over 30 min for HHI +19%, NHI +22%, HLI +16%, NLI +16%; max. O2-uptake for HHI +10.3%, NHI +4.0%, HLI +10.6%, NLI +11.0% ($p<0.05$; Wilcoxon test). Conclusion: 1. Endurance training under simulated altitude compared to N-training of the same relative intensity has no additional effect on the performance in normoxia. 2. Training at a low intensity has not much less effect than high-intensity training. First results of m. vastus lat. biopsies indicate: 1. Total volume of mitochondria increased to the same level with HI-training under N as well as under H. 2. Determination of mRNA concentration of cytochrome oxidase I and IV in the biopsies of the NHI-group indicate that at least part of the increase in mitochondria can be attributed to increased mRNA coding for mitochondrial protein.

M09

MECHANISM OF INCREASE OF LENS FLUORESCENCE WITH AGING

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Cataractous lenses contain a fluorescent substance, which is accumulated with aging. In the former methods used for isolation of this substance from the lens a strong alkali or acid were used which could have led to the modification of the substance. In these studies, we describe the isolation of the fluorescent lens substance under mild conditions, and we give its chromatographic characteristics.The fluorescent lens substance isolated from human lenses had the same molecular mass as that of xanthurenic acid when determinated by FAB. The fluorescent substance was identical with the xanthurenic acid derivatives in 5 different solvents used as mobile phase for HPLC. An accumulation of xanthurenic acid in cataractous lenses appeared to be the main cause of the increase of lens fluorescence observed in cataracts. The xanthurenic acid is the product of 3-hydroxykynurenine transamination by kynurenine aminotransferase.

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M10

Improved Biodistribution using a Trivalent Antigen-Binding Construct.

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We have prepared a trivalent antigen-binding construct formed from three Fab' fragments derived from the anti-CEA murine monoclonal antibody MAb35. The construct was generated using polyoxime chemistry. This approach leads to a homogeneous construct which retains full immunoreactivity. A comparison of the mono-, di- and trivalent $F(ab')_n$ materials *in vitro* revealed the expected trend of increasing association constant with increasing valency. The *in vivo* biodistribution of the ^{125}I-labeled trivalent construct was studied in xenograft-bearing nude mice. The construct showed tumor-to-blood ratios up to ten-fold higher than those seen for the parent antibody and ratios of tumor-to-normal-tissue accumulation were generally greatly improved. These improvements were achieved despite only modest reduction in maximal tumor accumulation when compared to the parent MAb35 and this augurs well for an improved potential for this novel construct as an agent for radioimmunotherapy and radioimmunoscintigraphy.

M11

TRANSLATION REGULATION BY SRP

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SRP is a cytoplasmic ribonucleoprotein implied in the transport of secretory and membrane proteins to the endoplasmic reticulum (ER). SRP recognises and binds specifically to nascent chains of secretory and membrane proteins early in translation and blocks then translation process before the ER membrane targeting of the complexe SRP/nascent chain/ribosome.SRP9, SRP14 and both extremities of SRP RNA molecule constitute a separated domain which has been shown to be responsible for the translation inhibition (elongation arrest). Using *in vitro* reconstitution experiment with deletion mutant mRNA of SRP9 and SRP14, we found that carboxy terminal part of SRP14 is necessary for the elongation arrest. A second approach has consisted in expression in bacteria and purification of different deletion mutants dimere SRP14-SRP9 (9/14-20C and 14/9-30C). Using this purified mutant dimere, we confirm the previous results: SRP14 carboxy terminal part doesn't affect the dimerisation with SRP9 and neither the binding with SRP RNA but abolish the elongation arrest activity of SRP.

M12

ISOLATION OF VERSICAN FROM U251MG GLIOMA CELLS

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Proteoglycans of the extracellular matrix are thought to play important roles in cellular processes like adhesion, migration and proliferation. The chondroitin sulfate proteo-glycan versican is a member of the large aggregating proteoglycan family. There are four alternatively spliced isoforms of versican currently known (V0, V1, V2 and V3). We recently identified the two largest splice variants V0 and V1 in the conditioned culture medium of the human glioma cell line U251MG. Versican-enriched fractions could be obtained from the spent culture medium by ion exchange and gel filtration chromatography. We are currently trying to isolate pure versican from these fractions in order to explore in the future the function of versican in *in vitro* and *in vivo* assay systems.

M13

SEQUENCE VARIATIONS IN LARGE-SUBUNIT RIBOSOMAL RNA GENE OF *Ammonia* (FORAMINIFERA, PROTOZOA) AND THEIR EVOLUTIONARY IMPLICATIONS.

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An unusually high divergence was observed in the ribosomal RNA genes of a free living population of foraminifera belonging to the genus *Ammonia*. The sequences of the large subunit (LSU) rDNA expansion segment D1 were obtained from 20 specimens named as *Ammonia sp. 1* and *Ammonia sp. 2*. The sequence divergence between the two species averages 35%. Within each species it ranges from 1% to 17.6% in *Ammonia sp. 1* and from 1.8% to 12.5% in *Ammonia sp. 2* respectively. We did not find two specimens having two identical sequences. In opposition to the generally accepted view, sequence variations were also found within a single individual. The variations among several rRNA gene copies in a single specimen may reach up to 14.9%. Most of the observed variations result from multiplication of CA or TA serial repeats occuring in two particularly variable regions. For single base changes, C-T transitions are most frequently observed.The variations of primary structure do not seem to interfere with the putative secondary structure model of expansion segment D1 in *Ammonia*. We discuss the evolution of expansion segments and their use for phylogenetic studies.

M14

The Use of rRNA Secondary Structure Information for Phylogenetic Reconstruction of the Insect Order Orthoptera

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Sequences from the two mitochondrial ribosomal RNA (rRNA) genes and the nuclear large subunit rRNA gene have been used to study the molecular systematics of the insect order Orthoptera. In phylogenetic reconstructions using the mitochondrial rRNA sequences, over 100 species have been surveyed and good resolution has been achieved between different families and subfamilies. In certain groups however, notably the superfamily Eumastacoidea and the family Acrididae, levels of multiple base substitution are high and this has inhibited phylogenetic reconstruction among closely related groups (e.g. subfamilies). We have attempted to improve the analysis in two ways. First, we have incorporated rRNA secondary structure into our analyses and examined correspondence between cladistic analyses of structural features and sequence derived phylogenies. Second, mitochondrial and nuclear based phylogenies have been compared in Acrididae. The results demonstrate that while certain structural features provide strong independent support for some phylogenetic findings, many types of structural variation (e.g. short deletions/insertions) are misleading if used in phylogeny reconstruction with an insufficient number of taxa.

M15

AGE-DEPENDENT CROSSLINKING OF CMP TO AGGRECAN

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Cartilage matrix protein (CMP) is a trimeric protein that has recently been purified and characterized under native conditions which allowed the proposal of a structural model (Hauser and Paulsson, 1994, J. Biol. Chem. 269, 25747-53). To examine the functional properties of CMP within cartilage extracellular matrix we identified its interaction with aggrecan. Fractions enriched in aggrecan were purified from bovine tracheal cartilage of different ages under native and dissociative conditions, respectively, and characterized by a combination of biochemical examination and electron microscopy. Cartilage matrix contains unbound CMP, a pool of CMP associated with aggrecan and a third pool of CMP strongly bound to the aggrecan core protein. CMP is attached via a nonreducible covalent crosslink of one of its subunits to the protein core. As a consequence, only 2/3 can be released even upon reduction under denaturing conditions. Electron microscopy revealed CMP bound to distinct sites in the extended chondroitin sulfate attachment domain E2 of aggrecan. The amount of CMP covalently bound increases with aging as seen by an increased decoration of E2 with affinity purified antibodies to CMP.

M16

POST-TRANSLATIONAL PROCESSING OF A VARIANT-SPECIFIC SURFACE PROTEIN IN *GIARDIA*

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The variant-specific surface proteins (VSPs) of the ancient protist *Giardia duodenalis* are cysteine-rich polypeptides that vary considerably in sequence and size. Analysis of one of these VSPs (CRISP-90) purified from a cloned *Giardia* isolate showed that this protein is post-translationally modified with both glycans and palmitic acid. The carbohydrate side chains represent a novel type of O-linked glycans composed of GlcNAc and Glc residues, and a palmitate residue is attached to the protein by an O-ester rather than a thioester linkage. During in vitro incubation of the cloned trophozoites, the membrane form of CRISP-90 (*m*CRISP-90) is specifically converted to a soluble isoform. This release of *m*CRISP-90 is associated with the cleavage of a carboxy-terminal peptide of about 3600 Da with the palmitic acid attached to this sequence. Fragmentation of the protein most likely occurs at a site directly preceding the hydrophobic membrane-anchoring domain of the molecule. It is hypothesized that processing of the membrane-associated VSP to its soluble isoform is an essential requirement for the ability of the parasite to undergo surface antigenic variation.