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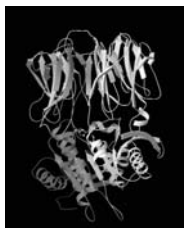

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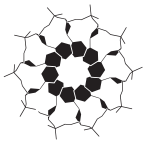

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Abstracts

P9 – FEBS Letters Young Scientist Award

O1-082
The role of Molecular Chaperones in protein folding

S. Vorderwülbecke[1], L. Ferbitz[2], T. Maier[2], N. Ban[2], B. Bukau[1] and **E. Deuerling**[1]
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A network of molecular chaperones supports the folding of newly synthesized proteins into their active conformation. In bacteria, the ribosome-associated chaperone Trigger Factor (TF) and the cytosolic DnaK chaperone system cooperate in the folding of newly synthesized proteins. The combined deletion of the TF encoding tig gene and the dnaK gene causes protein aggregation and synthetic lethality at 30°C. This synthetic lethality of Δtig ΔdnaK cells is abrogated either by growth below 30°C or by overproduction of the cytosolic GroEL/GroES chaperone system indicating a robust functional network of chaperones, which acts during the folding of newly synthesized proteins.
Whereas the cytosolic DnaK and the GroEL/ES-systems are well understood, the mechanism of action of the ribosome-associated TF chaperone was enigmatic. Recently, the crystal structures of the *E. coli* TF and of its N-terminal domain bound to the 50S ribosomal subunit of *H. marismortui* were solved. The results revealed that the three domains of TF are organized like a crouching dragon with an N-terminal „tail" binding the ribosome, the middle domain building the „head" of the dragon, and the C-terminal domain forming a „body" with two extended „arms". Remarkably, the N-terminal ribosome binding tail together with the C-terminal body forms a large cradle with a hydrophobic interior. This cradle bends over the ribosomal tunnel exit and in complex with the ribosome could provide a protected folding environment for newly synthesized proteins.

P10 – FEBS Journal Prize

O1-110
High level functional expression of diverse types of integral membrane proteins by an individual cell-free expression system

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Functional and structural studies of integral membrane proteins (IMP's) have been limited for a long time by insufficient production rates in the conventional bacterial or eukaryotic expression systems. Modified cell-free expression systems open now a new field for the study of this important class of proteins. We demonstrate the high level cell-free expression of a comprehensive variety of functionally folded IMP's including bacterial á-helical transporters, β-barrel-type porins and eukaryotic G-protein coupled receptors (GPCRs). Production rates as high as 6 mg per a single ml of reaction can be achieved. Cell-free produced IMP precipitates can easily be resolubilized with suitable detergents without any prior denaturation and refolding steps. In addition, the cell-free system is considerably tolerant for a wide selection of detergents and the IMP's can thus alternatively be produced directly as soluble proteins inserted into micelles of the preferred detergent. We present the functional analysis of a diverse series of cell-free expressed IMP's and we have evaluated the optimal cell-free expression conditions in order to obtain the highest specific activities. The labeling of proteins with stable isotopes is a prerequisite for their structural analysis by NMR and cell-free produced IMP's can be efficiently labeled without any background problems. The generation of NMR ready IMP samples is therefore routinely possible in less than 2 days. We present the rapid NMR assignment of the multispan tellurite transporter TehA by a new combinatorial approach using the cell-free amino acid specific labeling technique. It should further be emphasized that the main components of the cell-free expression system are relatively easy to prepare and the technique could be set up in almost each averaged biochemical lab.

A3 – Bioinformatics

O1-183
Multiple weak hits confuse transcriptional regulatory networks

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Robust systems, like the molecular networks of living cells are often resistant to single hits such as those caused by high specificity drugs. Here we show, using various attack strategies that partial weakening of the *Escherichia coli* and *Saccharomyces cerevisiae* transcriptional regulatory networks at a surprisingly small number (3 to 5) of points can be more efficient than the complete elimination/inactivation of a single node. These results may help to explain why broad specificity, low affinity compounds are often more efficient than their high affinity, high specificity counterparts. Multiple but partial attacks mimic well a number of *in vivo* a scenarios and may be useful in the efficient modification of other complex systems.

References

1. Ágoston V, Csermely P & Pongor S. Weak hits confuse complex systems. *Physical Review E* 2005; **71**: 051909.
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3. Ágoston V, Cema ar M, Kaján L & Pongor S. Graph representation of oxidative folding pathways. *BMC Bioinformatics* 2005; **6**: 19.

A4 – Human Genomics and Diseases

O1-178
Endophenotypes related to the Dopamine D4 receptor polymorphisms

M. Sasvari-Szekely[1], A. Szekely[2], Z. Csapo1, J. Gervai[3], Z. Tarnok[4], J. Gadoros[4], Z. Nemoda[1], C. Barta1 and Z. Ronai[1]
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The Dopamine D4 receptor (DRD4) gene is in the focus of psychogenetic studies since an association between Novelty Seeking and the 'long' DRD4 gene was shown in 1996. Further studies demonstrated a highly polymorphic chromosome region at the DRD4 gene, as well as a more complicated picture of the DRD4 related phenotypes.
Beside the DRD4 VNTR, several single nucleotide polymorphisms (SNPs) and a duplicated region (120 bp dup) have been shown in the DRD4 promoter. Numerous novel methods have been developed in our laboratory to avoid genotyping errors as a consequence of high density of SNPs and to determine the promoter haplotype. Moreover, molecular effects of promoter haplotypes have also been analyzed in a luciferase reporter system.
The DRD4 gene has been related not only to personality dimensions but also to attention deficit hyperactivity disorder, a common child psychiatric problem. Moreover, we found significant association between the infant disorganized attachment and certain DRD4 haplotypes. Our further studies on both clinical and non-clinical human populations show a significant association between the 'Persistence' personality dimension of human temperament and the 'long' DRD4 gene variant.
We hypothesize that low Persistence scores reflect insufficient inhibitory processes, which may also lead to deficiencies in sustained attention. To test this hypothesis, we analyzed a cognitive performance tasks using objective behavioral measures. Using an internationally standardized reaction time assay, a statistical association was shown between sustained attention and the 'long' DRD4 variants, pointing to a well defined, cognitive endophenotype. (This work was supported by the National Hungarian funds NKFP 0008/2002.)

C4 – Lipid-Protein Interactions in Membrane

O1-180
Computer simulations of lipid membranes

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Biological membranes are enormously complex in terms of both structure and dynamical properties. Even simple model system of membrane, consisting of lipid bilayers only, pose formidable challenges to experimentalists and theoreticians, due to the extremely large number of particles and to the forces between amphiphilic surfaces, which arise from both entropic and enthalpic factors. The most accurate method of calculating the dynamics of a many molecules system is to integrate the Newton equations of motion for all the atoms assuming a certain potential energy function, i.e. a force field. Its major drawback is that it often provides far more details of small-scale fluctuation motion of atoms than necessary for an understanding of many physical processes. We are using a variety of computational techniques such as molecular dynamics, dissipative particle dynamics and interface dynamics, to bridge the gap which separates phenomena of biological interests and computational accuracy. We present simulation of lipid membranes which have been validated experimentally *via* X-ray diffraction and AF microscopy, and we will discuss the effects of small molecules on the physical state of the membranes. Finally, some recent results on the spontaneous raft formation will be presented.

L1 – Protein Diagnostics, Protein Determination

O1-177
Reference methods and materials in standardisation and quality assurance

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Measurements in the health-related fields, like biomolecular protein marker determination are often seriously hampered by the lack of comparability of laboratory data. Contradictory measurement results are obtained due to a lack of traceability, commutability of reference materials (RMs), and assay calibration. The role of reference measurement systems and reference materials in standardisation and quality assurance is addressed in this presentation.
An example is given for the quantitative determination of human myoglobin in a human serum matrix, introducing a new combined experimental and bioinformatics approach to reliably quantify the myoglobin content of serum samples. In addition, myoglobin was used as internal standard to account for fluctuations in peak areas due to sample preparation and mass spectrometric detection. Myoglobin was separated from the highly abundant serum proteins by means of strong anion-exchange chromatography. Subsequently, the myoglobin fraction was trypsinized and analyzed by high-performance liquid chromatography-electrospray ionization mass spectrometry. The raw data acquired by the instrument was analysed automatically using a newly developed algorithm that detects and quantifies all ions belonging to peptides in the sample. The developed algorithm then computes a linear regression and confidence interval for the series of additive measurements with and without internal standard. We could quantify the amount of myoglobin using several tryptic peptides in parallel in a completely automated fashion. The relative errors observed were as low as 2.5%. This quantitative method could facilitate further absolute or relative quantitation of even more complex peptide samples, such as in the field of protein diagnostic markers.

O1 – Posters with late abstract submission

O1-001P
The nondiscriminating aspartyl-tRNA synthetase of *Pseudomonas aeruginosa*: residues involved in $tRNA^{Asn}$ recognition, and probing of its active site with the inhibitor aspartol-AMP

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In many organisms, the formation of asparaginyl-tRNA is not done by direct aminoacylation of tRNAAsn, but by specific tRNA-dependent transamidation of aspartyl-$tRNA^{Asn}$. This pathway involves a non-discriminating aspartyl-tRNA synthetase (ND-AspRS), that charges both $tRNA^{Asp}$ and $tRNA^{Asn}$ with aspartic acid. Recently, it has been shown for the first time in an organism (*Pseudomonas aeruginosa* PAO1) that the transamidation pathway is the only route of synthesis of Asn-$tRNA^{Asn}$, but does not participate in Gln-tRNAGln formation [Akochy *et al*. (2004) *J. Bacteriol*. 186: 767-776]; *P. aeruginosa* PAO1 therefore has a non-discriminating AspRS.
We report here the identification of two residues in the anticodon-recognition domain (H31 and G83) of *P. aeruginosa* ND-AspRS which are implicated in the recognition of $tRNA^{Asn}$: the two variants H31L and G83K have increased specificities of $tRNA^{Asp}$ charging over that of $tRNA^{Asn}$ by 3.5-fold and 4.2-fold, respectively. Thus, we show these residues to be determinants of the relaxed specificity of the non-discriminating AspRSs.
Aspartol-AMP, a stable analogue of aspartyl-AMP, is a competitive inhibitor of the discriminating AspRS of *Escherichia coli* [Bernier *et al*. *Bioorg Med Chem* 2005; **13**: 69-75]. The inhibition by aspartol-AMP of the aspartylation of unfractionated tRNA from *P. aeruginosa* by *P. aeruginosa* ND-AspRS is biphasic, and can be described by the sum of two competitive mechanisms. This biphasicity is explained by the fact that the aspartylation of pure $tRNA^{Asp}$ and of pure $tRNA^{Asn}$ are inhibited with Ki values of 41 µM and 215 µM, respectively. These results reveal that the two tRNA substrates of ND-AspRS interact differently with its active site.

O1-002P
Protein sources for developing blight resistance in legume *Cicer aritinum*

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Detailed analyses of extracts from several plants and microbial species have revealed antifungal activities against *A. rabiei*; the most potent antifungal activity was from *Bacillus cereus*. Purified barley ribosome inactivating proteins (Rip30), and chitinase (Chi26) in agar plate and microtiter well plate assays showed that the fungicidal effect of Chi26 and Rip30 was synergistic.
Moreover, extracts of local chickpea plant cultivars C44, CM72 and 6153 demonstrated the presence of internal antifungal activities against *Aschochyta rabiei*. The antifungal activity was present at constitutive and inducible levels when plants were grown either in the field or under laboratory conditions. The level of antifungal activities reflected the cultivar's response to blight resistance. Immunological studies showed the presence of a 27kDa inducible chitinase-like defense protein in infected chickpea pods and stems as evidenced by Western blots that employed anti-poplar chitinase antibodies. Molecular analyses of chickpea genome when detected through hybridization studies carried out under different stringent conditions indicated the presence of chitinase, glucanase and Rip-like gene family of defense genes with barely detectable mRNA levels. These studies have provided evidence that the presence of weak endogenous antifungal agents in chickpea can be augmented by the presence of foreign antifungal gene products through their integration into the chickpea genome.

O1-003P
Only one out of the two encoded genes accounts for the essential character of N-myristoylation in early embryonic development of *Arabidopsis thaliana*

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N-myristoylation is an irreversible protein modification catalyzed by N-myristoyl transferase (NMT) known to affect the membrane binding properties and activities of crucial proteins belonging to signal transduction cascades. One essential NMT gene has been described in fungi whereas two distinct functional genes are expressed in animals (HsNMT1 and HsNMT2 in humans). Two putative NMT genes could also be identified in *Arabidopsis thaliana*. Characterization of KO mutant lines of either gene indicated that AtNMT1 was essential for early embryonic development before the heart stage. In contrast, AtNMT2 was dispensable. A transgenic plant line featured by an inducible AtNMT1 gene was constructed in the nmt1 background to study further the impact of AtNMT1 expression at later developmental stages. AtNMT1 proved to be required for proper leave, flower bud, or fruit development. Together with promoter-GUS fusions and real-time PCR studies, the data indicated that AtNMT1 behaves as a strongly expressed housekeeping gene whereas AtNMT2 expression is much weaker and inducible. If expressed at normal levels, only AtNMT1 restored the developmental defect of the *Arabidopis* nmt1 KO line, compared to AtNMT2 or HsNMT2, suggesting distinct substrate specificity of the corresponding NMT. Accordingly, AtNMT1 but not AtNMT2 modified *in vitro* a subset of proteins known to be N-myristoylated. AtNMT1 is therefore responsible for the most crucial events associated to N-myristoylation in *A. thaliana*. These data will be discussed in the context of the possible cellular targets associated to each NMT. This analysis is made possible by the recent annotation of the complete N-myristoylated proteome associated to AtNMT1 activity in *A. thaliana*.

O1-004P
Apo E binding to the ligand binding domains of LRP

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The low-density lipoprotein receptor-related protein (LRP) is a member of a family of cell surface receptors that are both structurally and functionally related to the low-density lipoprotein receptor (LDLR). LRP is composed of two chains, the extracellular domain has four clusters of cysteine rich domains that resemble the LDL-receptor. Each cluster has 2-11 complement repeats followed by 1-4 EGF homology domains. LRP1 is responsible for clearing some 30 known ligands. Solution binding assays show that the two peptides, apoE(130-149) and apoE(141-155)2, interact with each of the sLRPs (2, 3 and 4). Surface plasmon resonance analyses of the sLRP/apoE peptide interaction show that both peptides bind the sLRPs with KD's in the 100 nM range, similar to the effective concentration required for observation of the cellular responses. Binding studies with smaller fragments composed of three complement repeats from each of the clusters have been carried out. Complement repeats CR15-17 from sLRP3 have the same binding affinity towards the ApoE peptide as the full length sLRP3. To study this interaction by NMR the ligand biding repeats have now been expressed in *E. coli* with the disulfide bonds reformed in the presence of calcium. Chemical shift perturbation reveals multiple complement repeats are involved in the interaction with ApoE. Progress towards the structure of the ApoE-LRP complex will be presented.

O1-005P
The structure of the myosin VI motor reveals the mechanism of directionality reversal

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We have solved a 2.4 Å structure of a truncated version of the reverse direction myosin motor, myosin VI, that contains the motor domain and binding sites for two calmodulins. The structure reveals only minor differences in the motor domain as compared to plus-end directed myosins, with the exception of two unique inserts. The first insert is near the nucleotide-binding pocket, and alters the rates of nucleotide association and dissociation. The second unique insert forms an integral part of the myosin VI converter domain along with a calmodulin bound to a previously unseen binding motif within the insert. This serves to redirect the effective "lever arm" of myosin VI, which includes a second calmodulin bound to an "IQ motif," towards the pointed (-) end of the actin filament. This repositioning largely accounts for the reverse directionality of this class of myosin motors. We propose a model incorporating a kinesin-like uncoupling/docking mechanism to fully explain the movements of myosin VI.

O1-006P
Selection of β-lactamase inhibitors by phage-display

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We have recently reported the selection of peptides specific for the active site of an anti-idiotypic antibody displaying a β-lactamase-like activity [Yribarren AS, Thomas D, Friboulet A & Avalle B. Selection of peptides inhibiting a β-lactamase-like activity *Eur J Biochem* 2003; **270**: 2789–2795]. One of the phage-displayed peptide (Pep90) issued from the selection procedures was shown to be a competitive inhibitor of the β-lactamase activity of the anti-idiotypic antibody, with a K_i = 38 µM. The submitted article reports the evaluation of the inhibition parameters of the selected peptide on *E. coli* β-lactamase and the corresponding anti-idiotypic catalytic antibody. We showed that pep90 is a competitive inhibitor of both catalysts, thus opening routes to the design of antibiotic-like molecules able to fight resistance of bacterial strains. We present our results since they provide evidence that a functional information could have been correctly transferred from a catalyst to its anti-idiotypic counterpart and moreover, they offer a completely new solution for the inhibition of ß-lactamase activity.

O1-007P
Regulation of the RNA polymerase II holoenzyme-associated Srb10/Cdk8

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Srb10/Cdk8 and its cyclin C subunit Srb11 function as a kinase component of the RNA Polymerase II holoenzyme mediator subcomplex, which phosphorylates the CTD of the largest polymerase core subunit and gene-specific transcriptional regulators during their interaction with the holoenzyme. CDK8 inhibits filamentous growth in yeast by phosphorylating the transcription factor Ste12. Here we demonstrate that nitrogen limitation causes disappearance of the CDK8 protein within four hours following a shift from rich medium to nitrogen limiting conditions. This effect appears to be transient, as CDK8 protein reappears by 12 hours and regains most of its kinase activity within 24 hours. Loss of CDK8 is mediated through control it its stability, as nitrogen limitation does not affect expression of its mRNA, and pulse chase labeling experiments indicate that protein stability is reduced from a half life of two hours in rich medium to one hour in nitrogen limiting conditions. CDK8-dependent phosphorylation on two known substrates, Gal4 and Ste12, are inhibited *in vivo* in cells growing in non-fermentable carbon, indicating that phosphorylation at these sites must also be inhibited in limiting carbon conditions. However, we observe only a minor decrease in CDK8 protein and kinase activity in cells shifted to non-fermentable carbon, and we also do not observe an effect on Srb11/Cyclin C expression. CDK8 protein was also stable in cells treated with a variety of additional nutritional and physiological stresses, indicating that nitrogen quality uniquely regulates its stability. Genetic analysis of upstream signaling mechanisms indicates that CDK8 activity is regulated by a combination of parallel signaling pathways responsive to nitrogen and carbon availability.

O1-008P
Haloperoxidase and HIV inhibition activities in marine sponges

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Combinatorial chemistry is an extremely powerful tool for drug discovery, although it cannot replace natural products as a source of chemical diversity. Natural products of marine origin are full of structural surprises and have a strong record of contribution to drug development. Within marine organisms,

sponges, with their peculiar characteristics, are one of the phyla that has attracted a lot of studies on natural products.
From a survey on sponge enzymes, *Erylus discophorus*, has shown the presence of haloperoxidase and HIV inhibition activities. Both molecules are very hydrophilic, with high molecular weights, over 200 kDa, and their isoelectric points are bellow physiological pH. The similarity of properties posed great problems in the isolation of the two different molecules. However, we are now gaining some insights in the extraction and early purifications steps, which are reported in the present work.
Sponges contain large amounts of microorganisms, embedded within the animal matrix, which can amount up to 40% of the biovolume. Using available molecular biology tools for community analyses in microbial ecology, we aim to discover whether these biological activities are due to the sponge itself or to some characteristic microbial community, specific for this species.

O1-009P
Elastin peptides modulate invasive potential of melanoma cells

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The incidence of malignant melanoma was significantly increased recent years and patients exhibit reduced life expectancy. The expression of matrix metalloproteinases (MMP), adhesion molecules and vascular endothelial growth factor (VEGF) in malignant cells are important steps during tumor progression.
We demonstrated the elastin protein at the invasion site of melanoma with histological and electronmicroscopical methods. And our immunohistochemical studies established that both superficial spreading melanoma and its cutaneous metastasis enhanced the level of VEGF, MMP-2 and MMP-3.
In this study, we present evidence that treatments with the chemotactic VAPG and VGVAPG elastin peptides increase cytoplasmatic level of MMP-2 and MMP-3, while decrease the expression of adhesion molecules (CD44, N-CAM and ICAM-1) and VEGF in two melanoma cell lines with different invasive potential. Immunocytochemistry, flow cytometry and quantitative real-time RT-PCR were applied to evaluate the changes of expressions.
In conclusion: interaction between phylogenetically conserved elastin sequences (VAPG, VGVAPG) and melanoma cells appears to be a significant point of tumor progression: (i) elastin and its fragments are potential substrates of MMP-2 and MMP-3, (ii) they have chemotactic effect on the melanoma cells, (iii) the cleaved soluble peptide fragments have the ability to increase the expression of MMP-2 and MMP-3, (iv) decrease the expression of adhesion molecules and (v) decrease the expression of VEGF.

O1-011P
Sphingosine kinase as a 'sensor' during chemotherapy-induced apoptosis in prostate cancer cells

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Ceramide and sphingosine 1-phosphate are interconvertible sphingolipids playing opposed roles in apoptosis. Ceramide functions as a pro-apoptotic molecule while sphingosine 1-phosphate exhibits anti-apoptotic properties, leading to the hypothesis that the balance between ceramide and sphingosine 1-phosphate levels determine cell fate. Here, we examined the involvement of the oncogenic sphingosine kinase, a critical regulator of the sphingolipid balance, in susceptibility to antineoplastic agents of prostate cancer cells.
Camptothecin, a known apoptosis inducer in LNCaP prostate cancer cells, is much less effective in PC-3 cell line. On the contrary docetaxel treatment caused massive loss of cell viability in PC-3, but had much lesser effect in LNCaP cells. Both docetaxel and camptothecin induced inhibition of sphingosine kinase and increase of ceramide/sphingosine 1-phosphate ratio only in cell lines sensitive to the drugs, but not in the more resistant ones.
Enforced expression of sphingosine kinase in PC-3 and LNCaP cells restored their resistance to chemotherapy, notably by decreasing ceramide/sphingosine 1-phosphate ratio, as it also decreased sensitivity of these cells to C2-ceramide (a cell-permeable analog that mimics endogenous ceramide effects). On the other hand, in both cell lines, both pharmacological sphingosine kinase inhibition and siRNA treatment induced profound apoptosis, which coupled with significant elevation of intracellular ceramide and loss of sphingosine 1-phosphate.
In vivo, docetaxel treatment of fluorescent PC-3 o.t. model established in nude mice induced cancer cells' apoptosis resulting in profound decrease of the volume of primary tumor and lymph nodes. Markedly, docetaxel treatment abrogated migration of cancer cells and formation of micrometastases. These events were coupled to sphingosine kinase inhibition and elevation of ceramide/sphingosine 1-phosphate ratio both in primary tumors and in lymph nodes.
Collectively our results show that apoptosis induction by chemotherapy in prostate cancer cells is correlated with sphingosine kinase inhibition. Ability of sphingosine kinase to determine the resistance of cancer cells to chemotherapy might propose its role as a responsive element in proapoptotic signaling. Thus sphingosine kinase inhibition might be a potential tool to sensitize resistant cancer cells to therapy.

O1-012P
The proteomic analysis of *Arabidopsis thaliana* nuclei and nuclear subfractions

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The nucleus is a key component of the eukariotic cell. On its territory, complex processes take place, e.g., DNA and RNA metabolism, all organized by the macromolecular nucleoprotein complexes, the chromatin. The elucidation of the chromatin components will allow a better understanding of development and differentiation. We have employed the standardized procedure for the nuclear isolation from the suspension *Arabidopsis* cell lines, one-and two-dimensional (2D) SDS-PAGE resolution of the protein mixtures, immunoblotting and microscopy to check purity, solubilization, fractionation and mass spectrometry. The ESI-MS/MS technique has been applied to identify the selected, cored spots from the 2D gels, or from fragments of the 1D gels. However, the majority of the identified proteins were revealed with a gel-less approach

by Multidimensional Protein Identification Technique (MudPIT). We analyzed 14 cored protein spots from 2D gels, 6 1D gel fragments, with on average 101 proteins in one fragment (up to 161 proteins in one fragment, 696 proteins in total), and 8 MudPIT runs, for the proteins isolated from whole cell nuclei, plantlets and from the nuclear fractions. During each MudPIT procedure we identified, on average, 280 statistically significant protein hits. Altogether, during all MudPIT runs, 1820 protein hits were uncovered. Among them were chromatin components (e.g., histones, histone deacetylases, the HMG proteins, nucleosome assembly proteins), the DNA metabolism proteins (e.g., transcription factors), ribosomal proteins, enzymatic proteins, nuclear and nucleolar matrix proteins (e.g., SAR DNA-binding proteins). The large group of proteins was formed by proteins described as "putative or unknown" (about 20% of all proteins). The bioinformatic analysis suggested a function for some of the hereto unknown proteins.

O1-013P
congenital disorders of glycosylation: biochemical diagnosis

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Congenital disorders of glycosylation (CDG) are genetic multisystemic diseases resulting from defects in the biosynthesis of glycoconjugates. [1] The most frequent type is CDG-Ia, which results from deficiency of phosphomannomutase (PMM2) that catalyzes the conversion of mannose-6-phosphate to mannose-1-phosphate in the cytosol. Our aim is to present our experience in the selective screening of CDG syndrome in a paediatric population (182 patients) with clinical suspicious of the disease, realised by a biochemical and genetic approach. A reliable quantitation of carbohydrate-deficient transferrin (CDT) as biological marker for diagnosis of CDG was defined, analysing serum transferrin glycoforms by HPLC. In all suspected cases detected in this way, enzyme activities were measured in leukocytes by means of a coupled enzyme system. Using this assay, the activity of PMM2 was found deficient in leukocytes from 5 patients with CDG-Ia. Molecular analysis of the corresponding PMM2 gene showed different mutations, confirming the diagnosis. Our data demonstrate that HPLC system, compared with immunoturbidimetry and isoelectric focusing methods, provides reproducible separation and quantification of the iron-saturated transferrin glycoforms with only small volumes of serum.

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O1-014P
CD8 is a kinetic promoter of ligand binding to the T-cell antigen receptor

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The mechanism of CD8 cooperation with the T-cell receptor (TCR) in the process of antigen recognition on live cells is suggested. The existence of two distinct populations of TCR and CD8 molecules on the cell surface emerges from two types of experimental results: Fluorescence correlation measurements yielded evidence for the existence of two populations of TCR and CD8 molecules with different lateral diffusion rate constants. Independently, evidence for such populations was derived from two distinct phases of association of the ligand, cognate peptide bound to major histocompatibility complex-encoded (MHCp) tetramers with the T-cells observed by flow cytometry. Based on these results a kinetic model for CD8-TCR cooperation is proposed. The model implies that the most efficient ligand association route corresponds to the fast ligand association with a raft-resident, colocalized CD8-TCR population. In this route, the reaction starts with MHCp association to CD8. This step effectively increases the probability of MHCp – TCR encounters and thereby promotes MHCp association with CD8 proximal TCR. The observed slow binding phase is assigned to ligand association with a non-colocalized CD8/TCR population. Taken together with results of functional assays of cytotoxicity, our data suggest that the colocalized, raft associated CD8-TCR population is the one capable of inducing the T-cell activation.

O1-015P
Peroxisome proliferetor-activated receptor-gamma and amyloid precursor protein processing

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The inflammatory response to amyloid beta (Abeta) accumulation has been proposed to contribute to the pathogenesis of Alzheimer's disease (AD) and to increase neuronal damage. This view is corroborated by a number of epidemiological studies providing evidence of a protective effect of long-term medication with nonsteroidal anti-inflammatory drugs (NSAIDs) against the neurodegenerative disorder [Rogers *et al*. (1993), Akiyama *et al*. (2000), Veld *et al.* (2001)]. These epidemiological studies are now well supported by recent data showing that *in vivo* treatment of APP transgenic mice with NSAIDs significantly diminished amyloid deposition [Lim *et al*. (2000)] and improved behaviour [Lim *et al*. (2001)]. Moreover, NSAIDs were shown to directly affect the generation of Abeta, suggesting a new mechanism of action behind the protective effect of NSAIDs [Weggen *et al.* (2001)]. A potential target of NSAIDs is the peroxisome proliferator-activated receptor gamma (PPARgamma) [Willson *et al*. (2000)], a ligand-activated transcription factor, member of the nuclear receptor superfamily.

Objective: Recent reports indicate that PPARgamma agonists downregulate Abeta generation, although the mechanism of this phenomenon still remains controversial [Sastre *et al*. (2003), Camacho *et al*. (2004)]. The aim of this study was to understand the effective role played by PPARgamma in the amyloidogenic process causing AD.

Methods: Human embryonic kidney (HEK293) cells stably transfected with APP695 were transiently transfected with human PPARgamma and its coactivator RXR. Abeta production, APP expression/ubiquitination and secretase activities have been analyzed by immunoblot. Cell death has been visualized with Annexin V-fluorescein isothiocyanate (FITC)/pro-

pidium iodide staining, under a fluorescence microscope.
Conclusion: We report that, in cultured cells, overexpression of PPARgamma dramatically reduced Abeta*f*nproduction, concomitantly increasing APP ubiquitination. Moreover, we demonstrate that the reduction of Abeta secretion protected the cells from H_2O_2-induced necrosis, suggesting a new mechanism at the basis of the anti-inflammatory properties of PPARgamma.

O1-016P
Serum and cerebrospinal fluid protein S-100b levels after severe head injury and their prognostic importance

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We compared serum and cerebrospinal fluid (CSF) S-100b protein levels after a severe head injury. These protein levels were investigated as indicators of brain damage for patients suffering from severe head injuries. The blood and CSF samples of 48 patients with Glasgow coma scale scores of 8 or below were taken within 1 to 11 hours after admission, then 24, 48, 72 hours after the injury. The outcome was evaluated 6 to 9 months after hospital discharge using the Glasgow Outcome Scale.
The overall mean serum S-100b concentration was 3.5-6.4 among the patients with unfavorable outcomes and 1.3-2.5 among the favorable outcome patients ($p>0.05$). The overall mean CSF S-100b concentration was 62.2-21.8 among the unfavorable outcome patients and 21.8-17.7 among those with favorable outcomes ($p<0.05$). These results indicate that CSF S-100b levels are clearly superior to serum S-100b levels for predicting outcome after severe head injury.

O1-017P
Structure and mechanism of RNA polymerase II CTD phosphatases

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Recycling of RNA polymerase II (Pol II) after transcription requires dephosphorylation of the polymerase C-terminal domain (CTD) by the phosphatase Fcp1. We report the X-ray structure of the small CTD phosphatase Scp1, which is homologous to the Fcp1 catalytic domain. The structure shows a core fold and an active center similar to phosphotransferases and -hydrolases that solely share a DXDX(V/T) signature motif with Fcp1/Scp1. We demonstrate that the first aspartate in the signature motif undergoes metal-assisted phosphorylation during catalysis, resulting in a phosphoaspartate intermediate that was structurally mimicked with the inhibitor beryllofluoride. Specificity may result from CTD binding to a conserved hydrophobic pocket between the active site and an insertion domain that is unique to Fcp1/Scp1. Fcp1 specificity may additionally arise from phosphatase recruitment near the CTD *via* the Pol II subcomplex Rpb4/7, which is shown to be required for Fcp1 binding to the polymerase *in vitro*.

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Kamenski T, Heilmeier S, Meinhart A, Cramer P. *Mol. Cell* 2004; **15**: 399-407.

O1-018P
8-Bromo-cyclic adenosine monophosphate induces activation of phospholipase D1 *via* ERK1/2 in human endometrial stromal cells

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We investigated the mechanism of 8-Br-cAMP-mediated phospholipase D (PLD) activation using a primary cell culture system of human endometrial stromal cells (ES cells). In ES cells, PLD activity was increased by the treatment with 0.5 mM 8-Br-cAMP, maximally at 5 min. To determine whether the effects of 8-Br-cAMP on PLD occurred as a consequence of PKC activation, ES cells were preincubated for 15 min with the PKC inhibitors, RO320432 (1 μM) and sphingosine (1 μM) or they were pretreated for 24 h with phorbol myristate acetate (100 nM) to down-regulate PKC. However, these treatments had no effects on PLD activation by 8-Br-cAMP. Subsequently, 8-Br-cAMP had no effects on the subcellular distribution of PKCα and PKCβI, conforming the lack of involvement of PKC. Moreover, 8-Br-cAMP activated ERK1/2, maximally at 5 min, and PD98059 (MEK inhibitor, 50 μM) and transfection of ES cells with dominant negative (DN)-MEK completely inhibited 8-Br-cAMP-induced PLD activation, suggesting that ERK1/2 mediates 8-Br-cAMP-induced PLD activation. To investigate the involvement of PKA, Src, and Ras in 8-Br-cAMP-induced PLD activation, we used PKA inhibitor, H89, and transfection of dominant negative Src and Ras. H-89 (10 μM) completely blocked 8-Br-cAMP-mediated PLD and ERK activation, implying involvement of PKA in this PLD activation. In addition, transfection of DN-Src or DN-Ras partially inhibited 8-Br-cAMP-induced ERK1/2 and consequently PLD activation, whereas cotransfection of DN-Src and DN-Ras compeletely inhibited ERK1/2 and PLD activation, suggesting that Src and Ras independently regulate ERK/PLD activation. Taken together, these results show a novel pathway in ES cells for activation of PLD by cAMP that is mediated through PKA/Src and Ras/ERK1/2 separately. Further, we investigated whether this activation of PLD by cAMP induces the decidualization of ES cells.

O1-019P
Influence of smoking on DNA damage and blood glutathione level

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Smoking habit is accepted as an important risk factor for certain types of cancer. Free radicals derived from smoking play an important role in the carcinogenesis by interacting with DNA. Susceptibility to oxidative stress and cancer is determined by the status of endogenous antioxidants which are protective against the harmful effects of free radicals. The aim of the present study was to evaluate the DNA damage and status of reduced glutathione (GSH) which is a powerful endogenous antioxidant in male smokers. 27 male smokers (smoking 20 cigarette per day) and 23 male nonsmokers were included by the study. They were not suffering from any disease. Venous blood was taken into heparinised tubes. Whole blood GSH level and DNA strand breakage frequency as an index for DNA damage in leukocyte DNA were measured. A spectrophotometric method and the Comet assay were used for the determination of GSH and DNA strand breakage frequency, respectively. Whole blood GSH level was found to be decreased. ($P<0.001$) but DNA strand breakage frequency was found to be increased ($P<0.001$) in male smokers as compared to controls. A negative correlation was determined between DNA strand breakage frequency and GSH ($r= -0.62$; $P<0.01$). It was thought that DNA damage is increased because of decreased GSH level in male smokers.

O1-020P
Role of heat shock proteins (HSPs) in tumor necrosis factor-α (TNFα) induced nuclear factor κB (NF-κB) signaling pathway

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Heat shock proteins (HSPs) are a group of proteins that have been implicated in various biological functions including the response to heat shock, oxidative stress and cytokine treatment. Under normal conditions, these proteins play important roles in cell function by changing protein conformation, promoting multiprotein assembly/disassembly, facilitating protein translocation and ensuring proper folding of the nascent polypeptides during translation. It has been suggested that in accordance with their roles under normal conditions, HSPs may also play crucial role in signal transduction by promoting folding or assembly/disassembly of signaling molecules.
Of this family, we have found HSP90 protein to be upregulated in a time-dependent manner starting from 15 minutes up to 4 hours by tumor necrosis factor-α (TNFα) treatment (20 ng/ml) in HeLa cells. Accordingly, electrophoretic mobility shift assay (EMSA) results have shown impaired nuclear factor-κB (NF-κB) binding to radiolabeled consensus oligo in presence of the specific HSP90 inhibitor-geldanamycin (GA: 0.5 μM) These results indicate that HSP90 plays an important role in TNFα induced NF-μB signaling pathway.
Having identified this, we want to proceed further to show protein-protein interactions of HSP90 and others by co-immunoprecipitation assays (co-IP), solving the interaction network upstream of inhibitor of κB kinase (IKK) complex. As a prerequisite to co-IP assay, we have accomplished to transfect HeLa cells with green fluorescent protein (GFP) with 95% efficiency *via* electroporation. In near future, by co-IP experiments, we aim to search for any possible cross-talks between NF-κB signaling pathway and other TNFα induced signaling pathways like JNK and p38 MAPK that might occur *via* HSP family.

O1-021P
Ecto-5′-nucleotidase (CD73) is a crucial mediator of vascular leakage *in vivo*

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Ecto-5′-nucleotidase (CD73) is a glycosylphosphatidylinositol (GPI)-anchored ectoprotein, which hydrolyzes nucleosides 5′-monophosphate to nucleosides, thus controlling the final step of the enzyme cascade that converts extracellular ATP into adenosine. Extracellular adenosine has been widely implicated in adaptive responses to hypoxia. Guided by previous work indicating that hypoxia-induced vascular leak is, in part, controlled by adenosine, we generated CD73 knock-out mice to test the hypothesis that CD73-generated extracellular adenosine functions in an innate protective pathway for hypoxia-induced vascular leak. cd73$^{-/-}$ mice bred and gained weight normally, and appeared to have an intact immune system. However, vascular leak was significantly increased in multiple organs, and following subjection to normobaric hypoxia (8% O_2), cd73$^{-/-}$ mice manifested fulminant vascular leak, particularly prevalent in the lung. Histological examination of lungs from hypoxic cd73$^{-/-}$ mice revealed perivascular interstitial edema associated with inflammatory infiltrates surrounding larger pulmonary vessels. Vascular leak secondary to hypoxia was reversed in part by adenosine receptor agonists or reconstitution with soluble 5′-nucleotidase, whereas it was intensified by CD73-specific inhibitors. In summary, these results identify CD73 as a crucial mediator of vascular leak *in vivo*, and therefore as a possible therapeutic target for disorders involving vascular leakage syndromes.

O1-022P
Epigenetic modulation of tumor-selective death signaling

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Altered chromatin function can lead to aberrant expression of growth regulators and may, ultimately, cause cancer. That many human diseases have epigenetic aetiology has stimulated the development of "epigenetic" therapies. Inhibitors of HDACs (HDACi's) show tumor-selective actions, induce proliferation arrest, maturation and apoptosis of cancer cells *in vitro* and *in vivo*, and are currently being tested in clinical trials. Intrigued by the tumor-selective action of HDACi's we investigated the corresponding mechanism(s) of action. We report that HDACi's activate tumor-selective TRAIL/Apo2L death signaling in acute myeloid leukemia (AML) cells and patients' blasts and define the corresponding mechanistic basis. The inductions of TRAIL, p21 and differentiation are independent HDACi activities with TRAIL expression being critical for the anti-tumor action of MS275 *in vivo*. HDACi's induced proliferation arrest, TRAIL-mediated apoptosis and suppression of AML blast clonogenicity irrespective of FAB status, karyotype and immunophenotype. No apoptosis was seen in normal CD34+ progenitor cells, indicating that TRAIL is the cancer-selective component in the HDACi action spectrum. Together with our results that the anti-cancer action of retinoic acid is mediated by the tumor suppressor IRF1 which mediates TRAIL promoter activation our studies identify TRAIL as a master mediator of the anti-cancer action of several signal transduction pathways and epigenetic drugs.
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O1-024P
Enhanced nuclear factor-κ B-associated Wnt-1 expression in hepatitis B- and C-related hepatocarcinogenesis: Evidence from functional proteomics analysis

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Chronic infections with hepatitis B and C viruses (HBV and HCV) are etiologically linked to hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC). Both may induce activation of nuclear factor κ B (NF-κ B) in hepatocytes that plays a crucial role in the regulation of cell growth and apoptosis.

Functional proteomics analysis of proteins associated with NF-κ B signaling complexes in both viruses-related HCC tumor and nontumor tissues may disclose possible common denominators in hepatocarcinogenesis. Using immunoprecipitation, two-dimensional polyacrylamide gel electrophoresis (2-DE), immunoblot analysis, and mass spectrometry, we analyzed NF-κ B associated protein complexes in four-paired human HCC tumor and nontumor tissues from HBV- and HCV-infected patients, respectively; and in one-paired tissue with dual viral infection. The quantity of NF-κ B-associated proteins was semi-quantitatively measured by protein spot intensity on the 2-DE gels. The results showed that enhanced expression (more than two fold) of NF-κ B-associated Wnt-1 protein was detected in tumor parts in three of the four-paired samples from HBV- and HCV-infected patients, respectively. The paired-sample from dual-viral hepatitis showed a highest increase (11.4 times) in the expression of Wnt-1 protein in the tumor part. These data could be further verified by immunoblot analysis of additional eight-paired HCC samples. In conclusion, our data suggest that enhanced expression of NF-κ B associated Wnt-1 protein may constitute one mechanism common to HBV-and HCV-related hepatocarcinogenesis. NF-κ B and Wnt-1 protein could be potential targets in designing highly effective therapeutic agents for the treatment of HCC and for chemoprevention of hepatocarcinogenesis.

O1-025P
Distinct trafficking defects of kidney anion exchanger 1 induced by dominant and recessive distal renal tubular acidosis mutants in polarized epithelial cells

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Distal renal tubular acidosis (dRTA) is an inherited kidney disease due to a defect of urinary acidification, that can be caused by dominant or recessive mutations occurring within the kidney anion exchanger 1 (kAE1), a glycoprotein expressed in the basolateral membrane of renal intercalated cells. The purpose of this study was to develop cellular models of dominant and recessive dRTA using polarized epithelial MDCK cells expressing normal or mutant forms of kAE1. Enzymatic deglycosylation, indirect immunofluorescence and FACS analysis showed that kAE1 was targeted to the basolateral membrane of the cells, reflecting the situation in kidney cells. In contrast, two dominant dRTA mutants (R589H and S613F), although properly folded, were retained in the ER, while two recessive mutants (G701D and S773P), despite their misfolding, were able to escape the ER and were either retained in the Golgi (G701D) or reached the basolateral membrane (S773P). Recessive mutants co-expressed with WT kAE1 could be targeted to the cell surface, while dominant mutants induced ER retention of WT kAE1. These results illustrate how dominant and recessive mutations induce dRTA, by altering in different ways kAE1 trafficking. (Supported by a CIHR Fellowship and Operating Grant).

O1-026P
Purification of copper/zinc superoxide dismutase from human erythrocytes and entrapped in multilamellar liposomes

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Copper/zinc superoxide dismutase (Cu/Zn SOD), plays a very important role in protecting organisms from oxygen toxicity. Thus as anti-oxidative stress mechanisms are localized in tissues and inside the cells, the limit of the defense mechanism of the body for any reason, (e.g., serious disease) may be induced. Also, it is proved that there is a relationship between the increase of superoxide anion and some diseases such as cancers, rheumatoid, arthritis and aging. Therefore, controlled manipulation of SOD at cellular level could be of importance as a therapeutic approach to these diseases. One of the possible ways for this controlled manipulation is the use of liposomes. Therefore in this study, the preparation of liposomes containing of Cu/Zn SOD purified from human erythrocytes was investigated.
Cu/Zn SOD was purified from human erythrocytes. Several methods, including, precipitation by acetone, chloroform, centrifugation and also ion exchange chromatography on DEAE-32 were applied. In this study, encapsulated SOD in multilamellar liposomes (MLV) was prepared using the film hydration method.
The results obtained from prepared human erythrocyte SOD was showed that at the end of the last stage, the purification was 21 times the result of the first stage, with a specific activity of 3000 units per mg (U/mg). The enzyme activity and retained enzymatic activity in MLV solution were 74±0.2 U/mg and 45%, respectively. Incubation of SOD-liposomes at 0°C and 37°C for 8 hours, caused enzyme activity to decrease to 66±0.2 U/mg and 31±0.2 U/mg, respectively. The results suggest that the encapsulation of Cu/Zn SOD in liposomes is a promising approach for the use of this enzyme as a therapeutic agent for the treatment of some diseases.

O1-027P
Activation of Proteinase-Activated Receptors promote human colon cancer cell proliferation through epidermal growth factor receptor transactivation

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Serine proteases have been known for a long time as proteolytic enzymes. Recent studies have shown that serine proteases act through membrane receptors (proteinase-activated receptors) to induce many biological responses. PARs are a new family of GPCRs which have a unique mechanism of activation. The receptor can be activated by an irreversible proteolytic cleavage in the N terminal domain thereby exposing a new N-terminal domain which interacts with the receptor and leads to receptor activation. Four members, PARs 1 to 4, have been cloned within the past decade. PAR-1, -3 and -4 are alternative thrombin receptor. PAR-2 is activated by typsin-like proteases and mast cell tryptase. We have shown recently that colon cancer cells express functional thrombin and trypsin receptors PAR1 and 2 which mediate a dramatic induction of cell proliferation and cell migration by these serine proteases [Darmoul *et al*. (2001), Darmoul *et al*. (2003)]. We analysed the mechanism whereby PARs promotes cell proliferation in human colon cancer cells. Using the HT 29 model cell line which expresses both PAR1 and PAR2, we show here that the activation of the ERK signal transduction pathway, as well as the PAR-dependent increase in cell proliferation, were dependent on the transactivation of the epidermal growth factor receptor (EGFR) and

involved EGF ligand release. PAR1- or 2-mediated cell proliferation was blocked by batimastat, a metalloproteinases inhibitor, EGFR neutralizing antibodies and the specific EGFR tyrosine kinase inhibitors AG1478 and PD 168393. We conclude that PARs-stimulated EGFR transactivation and subsequent ERK activation is mediated by EGFR ligands released through a metalloproteinase-dependent pathway. These studies identify the mechanism whereby PAR1 and PAR2 exert their proliferative effects in human colon cancers and document for the first time the cross-talk between serine proteases and growth factors in colon tumor growth.

O1-028P
Expression and characterization of recombinant nitric oxide synthases from *Physarum polycephalum*

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The *Physarum polycephalum* macroplasmodium is a single multinucleated cell which undergoes differentiation into sporangia under defined conditions. Previously, we found that during the starvation phase which is necessary to obtain sporulation competence, expression of nitric oxide synthase (NOS) is strongly induced and functionally related to formation of sporangia. Purification and cloning of *Physarum* NOS, the first non-animal NOS, showed that *Physarum* expresses two highly similar isoforms of NOS, termed physnosa (1055 amino acids) and physnosb (1046 amino acids), which both lack the spacer sequence conferring calcium dependence thus resembling the inducible NOS (NOS2) from animal cells [1]. Here, we recombinantly expressed both *Physarum* NOSs in *Escherichia coli* using a bicistronic vector allowing for coexpression of *Physarum calmodulin*. This expression was only successful if amino acids upstream the putative start methionine were included. As characterized for physnosa, 11 additional amino acids were sufficient for full activity of the enzyme. Biochemical characterization of partially purified recombinant enzymes showed that physnosb had a higher affinity for L-arginine (about 3-fold) and for tetrahydrobiopterin (about 10-fold) as compared to physnosa whereas the requirement for the other cofactors FAD, FMN and NADPH was comparable. In addition, we also characterized the genomic structure of physnosb using PCR and library screening techniques. Physnosb consists of 25 exons, physnosa is not yet fully characterized but appears to contain 27 exons. While intron/exon boundaries seem to be conserved between the two *Physarum* NOS genes, this is not the case when comparing with animal NOS genes. In summary, *Physarum* expresses two highly related NOSs, encoded by similarly structured NOS genes. Although both NOSs appear to be expressed in parallel, their different affinity for L-arginine and tetrahydrobiopterin, which is required for L-arginine binding and electron flux, suggests that in the intact cell physnosb may account for NOS activity in conditions of limiting substrate and/or cofactor concentrations.

Reference

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O1-029P
PITK, a PP1 targeting subunit that controls the activity of the transcriptional regulator hnRNP K

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Protein phosphatase-1 (PP1), through interactions with substrate targeting subunits, plays critical roles in the regulation of numerous cellular processes including RNA processing. Herein, we describe a newly identified regulatory subunit (PITK; Phosphatase Interactor Targeting K protein) that specifically targets the catalytic subunit of PP1 to nuclear foci to selectively bind, dephosphorylate and control the transcriptional repressor activity of heterogeneous nuclear ribonucleoprotein K (hnRNP K). Co-expression of PITK and hnRNPK in mammalian cell lines was shown to selectively affect AP1- and STAT3-mediated transcription. Additionally, PITK is phosphorylated *in vivo* at S1013 and S1017, residues that flank or reside within the PP1C-binding motif, and this phosphorylation negatively regulates the binding of the phosphatase to PITK. A mutant PITK with Ser-Ala substitutions at S1013 and S1017, when expressed in intact cells, was found to enhance native PP1 binding and elicited a more profound effect in the selective dephosphorylation of hnRNPK than wildtype-PITK. Taken together, our findings provide a mechanism by which transcriptional repression by hnRNP K can be discretely controlled through the regulation of PP1 activity.

O1-030P
Effect of oxidation on structure and function of neuroserpin, changes in fluorescence intensity and inhibitory activity

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Oxidative modifications of proteins attract considerable interest in neurodegenerative diseases. Under ischemic conditions, production of free radicals increases. Intracerebral administration or over expression of neuroserpin, an axonally secreted serpin after stroke decreases stroke volume in the area of ischemic penumbra. Cyclic oxidation and reduction of methionine residues acts as a scavenger of free radicals. The oxidation, as a common phenomenon can affect the structure and function of neuroserpin (high methionine residue content).

Methods: *E. coli*, BL21 and M15 strains were transformed with the plasmid, PQE31 containing the cDNA for human neuroserpin. After induction by IPTG, expressed protein was purified by Ni-SP50, a metal chelating resin precharged with 0.1 M $NiSO_4$. Neuroserpin was refolded in buffer ($NaH_2PO_4$20 mM, NaCl 100 mM, pH 7.8, 4°C). Inhibitory activity of neuroserpin was assayed by single-chain tPA from human melanoma cell culture and its chromogenic substrate, H-D-Ile-Pro-Arg-PNA-2HCl (λ_{max}: 400 nm). Purified human neuroserpin (500 µg) were incubated for 2 hours at room temperature in 1 ml reaction mixture containing 50 mM potassium phosphate, 100 mM potassium chloride, 1 mM magnesium chloride, 30 mM H_2O_2 at pH 5. Carbonyl groups were assayed by 2,4-dinitrophenylhydrazide 10 mM (λ_{max}: 366 nm). Fluorescence intensity (20 µg of neuroserpin) was assayed by spectrofluorimeter (excitation wavelength: 334 nm, emission wavelength: 363 nm).

Results: Carbonyl content was measured 4.1 ± 0.3 nmol/mg proteins. Oxidation of neuroserpin decreased its inhibitory activity up to 40%. Fluorescence intensity increased from 209 to 537 during 110 min incubation in reaction mixture buffer at room temperature, as compared with native form of neuroserpin.

Conclusion: In neuroserpin (a high methionine residue content protein) oxidation of these residues has considerable effect on its structure and function.

O1-031P
The TolC protein from Sinorhizobium meliloti is part of an ABC transporter involved in the biosynthesis of galactoglucan.

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The exopolysaccharide galactoglucan (EPS II), produced by the soil bacterium *Sinorhizobium meliloti*, is involved in the establishment of a symbiotic interaction between this bacterium and the leguminous plant *Medicago sativa*. In this interaction, bacteria fix nitrogen inside the plant roots in specialized organs called nodules, thus providing the plant with its own nitrogen source. EPS II biosynthesis is directed by the exp gene cluster, and one of these genes, encoding ExpE1, is essential for EPS II production [Moreira *et al. Microbiology* 2000; **146**: 2237]. ExpE1 is a calcium-binding protein secreted to the medium by an ABC transporter. The gene encoding an outer membrane protein of this transporter is not within the exp gene cluster. Homology searches using the gene sequence which encodes for the outer membrane protein TolC from *Escherichia coli* revealed the presence of a similar gene in the genome of *S. meliloti* 1021. The disruption of the *S. meliloti tol*C gene led to the non-mucoid and Fix- phenotypes. HPLC analysis of the supernatant of this mutant strain showed only vestigial presence of extracellular EPS II. Using an antibody against ExpE1, it was seen that this protein is produced but not secreted in the absence of TolC. This result is currently being further confirmed by complementation experiments with the *tol*C mutant. The ability of this mutant to efficiently infect *M. sativa* is being monitored using fluorescence and confocal microscopy. By proving the involvement of TolC in the secretion of ExpE1, the above results implicate TolC protein in the biosynthesis of EPS II by *S. meliloti*. (Supported by FCT – POCTI/AGG/39533/2002 and BME/44441/2002.)

O1-032P
Intracerebroventricular leptin modulates lipogenic genes expression in liver *via* SREBP-1c. Effects of aging.

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Leptin lowers adiposity and avoids lipid deposition in non-adipose tissues. Nevertheless, aging in human and rat is characterized by augmented adiposity and plasma leptin, suggesting leptin-resistance. SCD-1, a key enzyme in lipid metabolism, is a well known target of leptin. Nevertheless, reports on centrally mediated leptin effects on SCD-1 have been scarce and the mechanisms involved are unknown. Here we studied the effects of a 7-day intracerebroventricular (ICV) leptin infusion (0.2 mg/day) on the expression of SCD-1, FAS and CPT-1, and on the triglycerides (TAG) fatty acid composition in liver from 3-month-old Wistar rats. To investigate if central leptin resistance impairs lipid metabolism, the same experiments were performed in 24-month-old rats. In young rats ICV leptin down-regulated hepatic SCD-1 mRNA, thereby decreasing the oleic acid proportion in the TAG fatty acid content in this tissue and lowering plasma TAG. Additionaly, ICV leptin reduced FAS and increased CPT-1 mRNA expression. We suggest these ICV leptin effects occur *via* down-regulation of hepatic SREBP-1c mRNA. However, upon aging, in spite of endogenous hyperleptinemia, hepatic SCD-1 and FAS mRNA expression do not change, CPT-1 mRNA is down-regulated, plasma and hepatic TAG contents are augmented and adiposity is increased. Moreover, centraly administered leptin was not able to modulate none of the analyzed enzymes in aged rats, confirmig their central leptin resistance. This study provides a deeper insight into the central leptin-induced changes in hepatic lipid metabolism, as well as in the associated abnormalities in this regard of the elderly.

O1-033P
Structural and functional characterization of a new ubiquitous invertebrate metallothionein from the spiny lobster *Panulirus argus*.

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Metallothioneins (MTs) are low molecular weight proteins, rich in cysteines and with a high capacity to bind metallic ions. Their precise biological role in mammals has not been established and in invertebrates the reports are scarce. Here we characterize a new ubiquitous metallothionein (MTPA) from the lobster *P. argus*. MTPA mRNA expression and induction by Cd was analyzed by semiquantitative RT-PCR in different tissue explants. MTPA mRNA was detected in hepatopancreas, intestine, nervous tissue and muscle. Although the highest expression was observed in hepatopancreas, the highest induction by Cd was obtained in nervous tissue. MTPA aminoacid sequence deduced from its cDNA is homologous to other crustacean MTs sequences, but has an extra Cys in the C-terminal domain, potentially implying a higher metal binding capacity. However, according to a preliminary model of the tridimensional structure obtained by homology with other known crustacean MTs structures, this Cys does not seem to be involved in metal binding, since its side-chain would be oriented to the outside of the molecule. In fact, by HPLC-RP-ESI-MS analysis, recombinant MTPA binds 6 Zn^{2+} ions per molecule, akin to other crustacean MTs. Additionally, MTPA-Zn^{2+} inhibits the electron transport chain in hepatopancreatic mitochondria, increasing thereby ROS production, meanwhile apo-form effects are the opposite. Nevertheless, MTPA protects against oxidative stress, since the stimulation of ROS production by MT-bound Zn is smaller compared to equivalent amounts of free Zn. This constitutes the first report on MT effects on mitochondrial function in invertebrates and agrees with the results described for mammals, suggesting a connection between MTs and the energetic metabolism.

O1-034P
Effects of RO 31-8220 on insulin-mediated signalling pathway in adipocytes from adult and old rats

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Adipose tissue of aged rats is resistant to many actions of insulin. Atypical protein kinase C (aPKC) and protein kinase B (PKB) are apparently required for maximal insulin stimulation of glucose uptake, which involves translocation of the insulin-responsive glucose transporter GLUT4 from intracellular sites to the plasma membrane. RO 31-8220, an inhibitor of atypical protein Kinase C (PKC) isoforms, has been used previously to block insulin-induced activation and autophosphorylation of atypical PKC-ξ/λ in rat adipocytes. The aim of this work was to investigate the effects of RO 31-8220 on insulin-stimulated glucose transport, GLUT4 translocation and PKB activity in adipocytes from adult and old rats, in order to determine whether impaired PKC-ξ/λ activation contributes to adipose tissue insulin resistance in aging. Adipocytes from male Wistar adult (3-month) and old (24-months) rats were preincubated with or without 20 µM RO 31-8220 for 30 min and then stimulated with 80 nM insulin for 10 min. After incubations, cells were fractionated by differential centrifugation to obtain plasma membrane (PM), cytosol and internal membranes (LM and HM). Our data show that RO 31-8220 inhibits insulin-induced glucose transport, as well as GLUT4 translocation to PM, in adipocytes from adult and old rats. In adipocytes from adult (but not old) rats GLUT4 is hold on to the HM fraction. Additionally, RO 31-8220 caused an increase in PKB phosphorylation and PKB activity in adult and, to a lesser extent, in old rats. These data suggest that atypical PKCs are implicated in GLUT4 translocation to plasma membrane and that PKB activity is not sufficient to promote insulin-induced glucose transport.

O1-035P
Cooperation between catalytic and non-catalytic nucleotide binding sites of a bacterial restriction RNase

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The $tRNA^{Lys}$ -anticodon nuclease PrrC represents a family of bacterial restriction RNases likely to act as second-strike antiviral deices. *E. coli* PrrC is kept inactive by association with the type IC DNA restriction endonuclease EcoprrI and is activated by Stp, the phage T4-encoded inhibitor of EcoprrI. An NTPase domain occupying the first two thirds of PrrC communicates the activating signal triggered by Stp to the C-proximal anticodon nuclease site. *In vitro* studies using cell free extracts have suggested that the activation reaction requires GTP hydrolysis by PrrC as well as avid binding of dTTP by the same protein [Amitsur *et al*. *Mol Microbiol* 2003; **50**: 129-143]. Isolation of PrrC, required for examining the proposed GTP/dTTP synergy more closely, has been enabled by means to overcome the self-limiting translation and thermal instability of this protein. These measures included the use of a more readily expressed PrrC mutant, the chemical chaperone trimethyl-amine-N oxide (TMAO) and the avid nucleotide ligand dTTP. They enabled purifying the active form of PrrC to near homogeneity, determining its oligomeric structure and confirming the proposed differentiation of its nucleotide-binding sites into high and low affinity subsets specific for dTTP and GTP, respectively. PrrC resembles in this regard rotatory ATPases but differs from them in being a tetramer rather than hexamer and in diversification of its nucleotide binding sites also with respect to the type of cognate nucleotide.

O1-036P
Studies on the correlation of *Mycoplasma pneumoniae* infection with various immunological and haematological findings in HIV infected patients with underlying upper and lower respiratory infections in Chennai, South India

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Mycoplasma pneumoniae has been implicated with community-acquired pneumonia and mild to severe respiratory infections in the normal population. The prevalence of this mollicute in HIV infected patients has never been reported from India. Mycoplasmas have also been reported to act as cofactors in AIDS progression.

Objectives:

a) To divulge the incidence of *Mycoplasma pneumoniae* and other respiratory pathogens in the respiratory specimens of HIV infected patients.

b) To compare the sensitivities of induced sputum and throat swab specimens for detecting *M. pneumoniae*.

c) To correlate the various haematological and immunological findings with infection due to *M. pneumoniae* in the HIV infected patients tested.

Materials and Methods: The present study has been carried out on 60 HIV infected patients presenting with underlying pulmonary complaints and whose clinical presentation was consistent with disease caused by *M. pneumoniae*, after obtaining informed consent subsequent to approval by the Institutional Review Board (IRB) on human ethics in Chennai where the recovery rates of *M. pneumoniae* from induced sputum and throat swab specimens of HIV infected patients were compared and the haematological and immunological findings were analysed. Patients screened were from the age groups ranging from 15 to 60 years, whose respiratory specimens were cultured on PPLO glucose agar and broth, the later with 1% methylene blue. Presumptive identification of *M. pneumoniae* was carried out using guidelines proposed by the Subcommittee on the Taxonomy of Mollicutes, 1979. The respiratory specimens from the HIV-infected subjects were later analysed for their recovery rates, incidence of other bacterial, fungal pathogens, AFB, Pneumocystis carinii and their correlation features with CD4+ and CD8+ lymphocytes were also analysed and compared.

Results and Conclusion: The male to female ratio of the study population was 51:09. The mean age of the patients was 39 years. *M. pneumoniae* was presumptively detected from 23 (38.3%) of the HIV infected patients. Induced sputum and throat swabs yielded 82.6% and 55% of the mycoplasma isolates respectively, which suggests that induced sputum can be the better specimen compared to throat swabs. Simultaneous positivity of both specimens was detected in 13 (56.5%) cases. Besides Candida spp (80%), *Staphylococcus aureus* (26.6%), *Streptococcus pneumoniae* (21.6%), *Pseudomonas aeruginosa* (18.3%), AFB (16.6%), *Klebsiella pneumoniae* (15%), *Moraxella catarrhalis* (8.3%), b-hemolytic streptococci, *P. carinii, M. fermentans* (6.6%), diphtheroids (5%), *A. fumigatus and E. coli* (1.6%) were the predominant isolates. The detection rate of *M. pneumoniae* was found to be high in patients with depleted CD4 levels. The mean CD4 count of the study cases was 106 cells/ìl, whereas the value was only 78 cells/µl among those positive for *M. pneumoniae*. The study shows that CD4 depletion may enhance mycoplasma infection in the respiratory tracts of HIV infected patients.

O1-037P
Regulation of neuregulin receptors during myogenesis

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Neuregulins are growth factors that induce myogenesis in a concentration-dependent manner, although a late myogenic character, the GLUT4 glucose transporter expression, is repressed at high neuregulin concentration. During myogenesis, neuregulin receptors, ErbB3 and ErbB2, are transiently increased. ErbB3 is essential for the myogenic process. After myotube formation, ErbB3 is exchanged by another neuregulin receptor, ErbB4 in the myogenic cell line 1C9. Exchange of ErbB3 by ErbB4 is reproduced in regeneration of soleus and EDL muscles. Adult muscle fiber contains ErbB2 and ErbB4 clustered at the synaptic site. ErbB3, although it is detected at the neuromuscular junction, belong to the pre-synaptic membrane compartment. Denervation of adult muscle fiber induce increases in ErbB3 and ErbB2 levels, that are not located at the synaptic site. Neuregulins regulate levels of their receptors. High neuregulins concentration (3 nM) induces ErbB3 expression, and reduces ErbB4 expression in muscle cells, whereas low neuregulin concentration (30 pM) has opposite effects. Neuregulin inhibits GLUT4 expression when it signals through ErbB2/ErbB3 whereas induces GLUT4 expression when it signals through ErbB2 ErbB4. In conclussion, neuregulins regulate myogenesis and in a receptor-dependent manner regulate the glucose transporter GLUT4 expression. ErbB3 is required at early stages of myogenesis to induce this process, whereas ErbB4 contributes to maintain late myogenic characters as the GLUT4 expression.

O1-038P
Proteome analysis of korean mistletoe lectin treated non-small-cell lung carcinoma cells

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A proteomic approach has been applied to investigate changes of protein expression level of non-small-cell lung carcinoma A549 cells following treatment with lectin from Korean mistletoe (*Viscum album L. coloratum*). Korean mistletoe lectin (KML) has been reported to inhibit effectively growth of lung carcinoma cells and also expression of the angiogenic factors on mRNA level. These mechanisms are unidentified. Through the isoelectric focusing (IEF) and two dimensional gel electrophoresis (2DGE), changes of protein expression level were profiled. Eighty spots were changed and among them, 11 spots were remarkably increased, 13 spots were decreased in quantity. Those differentially expressed 24 spots were analyzed *via* matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF-MS). We confirmed that some proteins interrelated with crucial survival factors of carcinoma cell, such as tumor necrosis factor-alpha (TNF-α), cytosolic inorganic pyrophosphatase, asialoglycoprotein receptor 1, centrosomal protein 1, creatine kinase, prohibitin and tubulin folding cofactor B, were altered. At the same time, some proteins that would be affected by KML were confirmed. Taken together, those quantitatively changed spots are might be related to anticancer mechanisms of KML and can be critical resources to reveal the interrelationship between KML and A549 cells. (Supported by Grant No. 204061-3 from ARPC.)

O1-039P
A novel continuous electrophoresis method for purification of lysozyme from hen egg white

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Lysozyme (mucopeptide N-acetylmuramoylhydrolase) is an ubiquitous enzyme with the ability to lyse certain bacteria by hydrolyzing the β-linkage between muramic acid and N-acetyl glucosamine of mucopolysaccharides in the bacterial cell wall. Lysozyme has tremendous potential in clinical application for treatment of ulcers, infection, wounds and as a potentiator of some antibiotics. The expanding potential for application of lysozyme in the many fields of sciences dictates the urgency for developing efficient and simple methods for lysozyme purification. Direct crystallization, ultrafiltration, ion exchange and affinity chromatography have been used to purify lysozyme from hen egg white. However severe limitation of these methods including lengthy steps, high cost and dilution of the egg white during processing have hampered their application.
The purpose of this study was to develop a simple, efficient and very low cost electrophoretic method for purification of lysozyme from egg white. 50 ml of hen egg white was homogenized eith equal volume of phosphate buffer (0.05 M, pH=8.6). 50 ml of this homogenized egg white was spilled into a beaker and the equal volume of phosphate buffer was spilled into the another same beaker. Traditional electrophoresis platinum electrodes was used. The anode electrode was put into the homogenized egg white beaker and cathode electrode was put into the buffer beaker. Solutions of two beakers were linked with five layers of whattman paper soaked with the phosphate buffer. Electrophoresis was performed with a 9-10 mA constant current. At the end of the study, lysozyme activity test and SDS-PAGE was performed in the nascent homogenized egg white and in the cathode solution.
Results showed that with the progress of time, egg white lysozyme was purified and concentrated in the cathode solution. After 5 hours the purification fold of lysozyme was 35.2 and lysozyme yield was 92%. SDS-PAGE results showed that the lysozyme separated by this method was 100% pure. In this method with the progress of time, lysozyme was concentrated in the cathode solution. In comparison with the other methods, that are multi steps and complex, this method is a single step and very simple and its efficiency is better. This method can be used in large scales for bulk purification of lysozyme from natural and recombinant sources.

O1-040P
SREBP contributes to the cell growth program activated by PDGF

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We analyzed the transcriptional program elicited by stimulation of normal human fibroblasts with platelet-derived growth factor (PDGF) using cDNA microarrays. We identified 103 significantly regulated transcripts that had not been previously linked to PDGF signaling. Among them, a cluster of genes involved in fatty acid and cholesterol biosynthesis, including stearoyl-CoA desaturase (SCD), fatty acid synthase and hydroxy-methylglutaryl-CoA synthase (HMGCS), was

up-regulated by PDGF after 24 h of treatment, and their expression correlated with increased membrane lipid production. All these genes are known to be controlled by sterol regulatory element-binding proteins (SREBP). PDGF increased the amount of mature SREBP-1, and regulated the promoters of SCD and HMGCS in a SREBP-dependent manner. In line with these results, blocking SREBP processing by addition of 25-hydroxycholesterol blunted the effects of PDGF on lipogenic enzymes and cell growth. SREBP activation was dependent on the phosphatidylinositol 3-kinase (PI3K) pathway, as judged from the effects of the inhibitor LY294002 and mutation of the PDGF β-receptor tyrosines that bind the PI3K adaptor subunit p85. Fibroblast growth factors (FGF-2 and FGF-4) and other growth factors mimicked the effects of PDGF on NIH3T3 and human fibroblasts. In conclusion, our results suggest that growth factors induce membrane lipid synthesis *via* the activation PI3K and SREBP, which contributes to cell growth.

O1-041P
Induction of EphA2 expression by epidermal growth factor-mediated signaling

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In a search for epidermal growth factor receptor (EGFR) regulated genes, the receptor tyrosine kinase EphA2 was identified. EphA2 belongs to the Eph receptor family, which is the largest group of receptor tyrosine kinases in higher eukaryotes. Elevated EphA2 expression is frequently found in cancer cells and is thought to play a role in angiogenesis and metastasis.
In the present study we have investigated the role of ligand activated EGFR and the cancer specific constitutively active variant EGFRvIII in the regulation of EphA2 expression. Our results show that activated EGFR and EGFRvIII induce an increase in mRNA and protein levels of EphA2 in a number of mammalian cell lines. This induction was found to be rapid and sustained. Using a panel of small molecule inhibitors we found that the induction of EphA2 expression was dependent on the EGFR/EGFRvIII tyrosine kinases and MEK signaling. Furthermore, the expression of EphA2 seemed to be dependent on cellular adhesion to extracellular matrix proteins, as cells forced to grow in suspension lost expression of EphA2. EphA2 levels in these cells were only weakly induced by EGFR activation. Immunoprecipitation experiments indicate that EphA2 associates with EGFR and EGFRvIII, however stimulation of cells with EGF or EphrinA1 failed to induce transphosphorylation of either receptor.
Our results thus indicate that EGFR is an important regulator of EphA2 expression and that EphA2 could be involved in EGFR mediated tumorigenesis.

O1-042P
Proteomics of cyanobacterium *Synechocystis* sp. PCC 6803. Identification of thylakoid membrane proteins

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Thylakoid membranes purified from the cyanobacterium *Synechocystis* sp. PCC6803 by a combination of sucrose density centrifugation and aqueous polymer phase partitioning were used in proteomic studies. Proteins from totally purified membranes were separated by two-dimensional gel electrophoresis (pH 4-7 and pH 6-11). Sixty-three different proteins were established by MALDI-TOF mass spectrometry analysis followed by database search using the Mascot program. Six of them were predicted to be integral membrane proteins.
In an attempt to enrich integral membrane proteins, the thylakoid membranes were washed with urea and used for one-dimensional gels. It allowed identify thirteen additional proteins, including eight predicted integral membrane proteins.
Among totally seventy-six different proteins identified in this work, twelve have a predicted signal peptide, of which eight are Sec signals and four are putative Tat (twin arginine) signals. Four proteins have a signal sequence motif characteristic for lipoproteins. Subunits of the well characterized thylakoid membrane constituents Photosystem I and II, ATP synthase, cytochrome b6-f complex, NADH dehydrogenase and phycobilisome complex were identified. One of the two Signal peptidases type I of *Synechocystis* was found in the thylakoid membranes, whereas the second one has been identified previously in the plasma membrane. Sixteen proteins are predicted to be "hypothetical proteins" with unknown function. Interestingly, more than half of these proteins have homologous genes found only in cyanobacterial genomes. In addition, novel thylakoid membrane proteins, both integral and peripheral, were identified, among these are also enzymes involved in pigment biosynthesis.

O1-043P
New fluorescence dyes for protein gel stains

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Advanced proteomic techniques require highly sophisticated means for the detection and analysis of biomolecules. Three new fluorescence dyes for the staining of proteins in polyacrylamide gels have been developed: LUCY-506, LUCY-565 and LUCY-569. These dyes were tested in different gel-systems, and applications for 1D- and 2D-Electrophoresis have been worked out. The following parameters were elaborated: sensitivity, detection-limits, linear dynamic range, background, specificity, photostability, pre- and post-electrophoretic staining protocols, electrophoresis conditions, time-optimization, ease of use, detection and imaging, and compatibility with subsequent MS-analyses.
Comparisons to current state-of-the-art dyes were drawn under different experimental conditions. The detection limits for the 3 dyes ranged between 3-10 ng/band. Linearity was given up to 1000-6000 ng/band and is therefore larger than for most silver stains [White *et al. Electrophoresis* 2004; **25**(17): 3048-3054], coomassie blue or other fluorescence dyes. The standard procedure is a post-electrophoretic stain without fixation, which is completed after 60 min. Using a modified staining protocol, it is also possible to stain a gel and perform a western-blot afterwards. Native gels can be visualized by rinsing the gel in SDS after the run, but before staining.
Several devices can be used for the detection, e.g., illuminating the gel on a transilluminator (Dark-Reader, UV-Screen) and imaging the gel using a CCD- or Polaroid-Camera. Alternatively a laser scanner can be employed, using the corresponding excitation and filter settings. Photostability of the dyes was determined by continuous UV-illumination of the gels and imaging on a laser scanner in defined time intervals. The new dyes offer a suitable alternative to current silver staining techniques or existing fluorescent staining methods for the detection of minute amounts of protein.

O1-044P
Oxidative modifications induced by the glycation of bovine serum albumin on its structure and on cultured adipose cells

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Non enzymatic glycosylation (glycation) and oxidative damages are major research areas because such modification of proteins have been well documented in numerous disease states. Non insulin dependent diabetes mellitus is dramatically associated with early occurrence of vascular complications, together with structural and functional alterations of albumin, which undergoes increased glycation.
It is well known that protein could switch after glycation or oxidation from beneficial compound to deleterious one and previous studies showed that albumin, which exerts important antioxidant properties, presented impairments in its beneficial activities after *in vitro* glycation or oxidation.
In this work, we first characterized structural damages induced by the glycation of bovine serum albumin (BSA). Then, we identified a pathophysiological effect of glycated BSA on primary cultures of human adipose cells as inducing an accumulation of oxidatively modified proteins. BSA has been incubated with 0, 5, 10, 25 or 100 mM of glucose at 37°C for 7 weeks. 5 mM and 25 mM correspond to the physiological and pathological concentrations of glucose, respectively. Enhanced BSA glycation percentages were determined, after the incubations with increasing concentrations of glucose, using boronate affinity columns. Occurrence of oxidative modification in the different preparations have been found in glycated BSA by measuring their free thiol groups contents, the relative electrophoretic migration on native gel and infrared spectrometry spectra.
Primary cultures of human subcutaneous adipocytes have been incubated in the absence or presence of native or glycated albumin. A carbonyls assay was used to determine oxidative modifications in cells proteins resolved on both one and two-dimensional gels and revealed an accumulation of carbonyl-modified proteins when the cells have been incubated with glycated albumin. We thus propose that oxidative modifications of adipose proteins in relation with the enhanced glycative phenomenon in diabetics might be involved in the increased mortality risk of these patients.

O1-046P
High throughput screening assays for bacterial primases

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Bacterial primase is a DNA-dependent RNA polymerase that functions at the replication fork to create short oligoribonucleotide polymers. Knockout and conditional mutant experiments have indicated that primase is an essential replication protein in three different bacteria. The essentiality of primase makes it a good target for novel antibiotic development. Since the bacterial primase has many features that differ from the eukaryotic primase, it should be possible to discover inhibitors that are specific to the bacterial primase and that don't cause side effects with the eukaryotic one. In order to screen libraries of compounds, we needed to develop a high throughput screening assay and a faster method to characterize the primase activity. For many years, the most common assay for measuring primase activity was to incorporate radiolabeled nucleotides into the primer, separate the different lengths of polymers by electrophoresis, and use various imaging methods to characterize them. Recently, one company created a high throughput screening method from this by using scintillation proximity beads instead of electrophoresis. That assay suffers from some correctible design flaws but we sought to develop other methods. One of the assays that we designed uses denaturing high pressure liquid chromatography to separate the RNA polymers according to length and sequence. It is much faster than the radioactive method and allows detailed analysis of product lengths and concentrations. The other assay that we have developed is a microplate-based fluorescence assay that does not use radioactivity and is adaptable to robotic screening methods.

O1-048P
Prediction of protein-protein interactions

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A protein–protein docking method has been developed for an initial stage of unrestricted docking. The method employs high resolution protein representation on fcc lattice and performs a rigid-body docking based on shape complementarity. Obtained ensembles of possible docked complexes with additional biological informations allow to identify the right complex and are a good starting point for the next docking stages: rescoring and introduction of flexibility.

O1-049P
Protein folding using sparse restraints: chemical shifts, residual dipolar couplings and theoretically predicted contacts.

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An *ab initio* prediction of protein structures could be enhanced with applying sparse, but easy to obtain, experimental data to protein folding simulations. Combining theoretical methods with practical experiments accelerates determination of protein structure without limiting the model resolution. In this work we present an application of the data from relatively simple NMR experiments to the ab-initio prediction performed by a simplified lattice protein model CABS [1] with a Replica Exchange Monte Carlo method.
We tested two kinds of the experimental data: chemical shifts (encoding the approximate local geometry of the backbone) and residual dipolar couplings (providing the long-range orientational information). Details of incorporating chemical shifts into the protein folding algorithm could be found in the recent publication [2]. Apart from the experiment based restraints, we also employed homology based methods: the protein secondary structure prediction and the prediction of the protein contacts. The simultaneous application of the experiment and homology based restraints significantly improved the prediction, especially in a case of small, topologically simple protein structures.

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O1-050P
Searching protein energy landscape with structural clustering.

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A new approach to investigate the distribution of the local energy minima on protein energy landscape is proposed. The CABS [1] (CAlpha, CBeta and the center of mass of the Side chain) reduced representation modeling tool was used to generate large sets of protein conformations. Obviously a different than CABS protein modeling algorithm could be used instead. Thus, the results are rather general. C? traces from an ensemble of models are hierarchically clustered with the HCPM [2] (Hierarchical Clustering of Protein Models) method.
It has been shown that our clustering procedure is able to recognize these areas in the conformational space of a protein that feature a high density of states. All configurations belonging to a certain cluster are very close to each other and have on average lower energy than neighboring structures. Thus, each cluster corresponds to a certain local minima on the energy landscape. For the purpose of composing a structural library they can be replaced by a single representative. While the spectrum of distances between clusters representatives is broad, they are well separated one from each other. The corresponding structures reflect the manifold of distinct topologies sampled during the simulations. Hence, our clustering procedure allows for creating libraries of decoys, containing a wide variety of low energy structures. Such organized libraries of decoys could be used as a tool for model selection and evaluation, in selecting protein fragments for fold asembly, in threading and in other applications realated to fold recognition and de novo protein structure prediction.

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O1-051P
Development of antiviral drugs targeting the flaviviruses fusion mechanism

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We have successfully identified and tested small molecules which inhibit the dengue virus fusion mechanism, a process essential for viral infection of cells. The compounds were identified by in silico docking of a compound library against the dengue virus envelope (E) protein. We hypothesize that these compounds inhibit the transition of the E protein from its metastable pre-fusion form to its stable post-fusion form. We tested the compounds in plaque and MTT assays and identified one compound with an IC_{50} of 1 μM with no mammalian cytotoxicity. This lead compound is now undergoing further studies for the development of a dengue virus fusion inhibitor. Furthermore, the high conservation of E protein structural elements amongst the flaviviruses suggests that this compound may also serve as a lead for the development of fusion inhibitors against other members of the flavivirus family, for example the West Nile fever and Japanese encephalitis viruses. Flaviviruses, in particular, dengue virus are a major public health problem in tropical and subtropical areas of the world. Up too 100 million people are infected annually and the incidence of these diseases is increasing over the last two decades. Infection with dengue virus can result in acute and potentially fatal haemorrhagic fever. Currently there are no therapeutic agents available for treating flavivirus infections. The identification of our lead compound is a first step toward their development.

O1-052P
Epigenetic code changes during early mouse embryo development – focus on histone deacetylation

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In eukaryotes chromatin structure is based on the nucleosome particle consisting an octamer histone core and 147 bp wrapped DNA. Histone N-terminal and C-terminal tails are subject to a large number of posttranslational modifications, e.g. acetylation, metylation, phosphorylation. These epigenetic changes (called reprogramming) are in focus of nuclear cloning studies. Preimplantation development covers the time period from fertilization to implantation where gene expression reprogramming is likely to be responsible for the transformation of the differentiated oocyte into the totipotent blastomeres.
There are little knowlegde how histone acetylation-deacetylation occurs in the molecular level, how are these processes regulated, which enzymes are involved during preimplantation development and how are enzyme substrate specificity controlled.
We first determined mRNA level of three histone deacetylases (HDAC1, HDAC2 and HDAC6) in individual mouse embryos from oocyte to blastocyst stage with our sensitive real-time RT-PCR method. We found that these three different HDACs showed totally different expression pattern.
In conclusion it is necessary to determine which of the known eleven HDACs and eleven histone acetyltransferases (HATs) are involved in the preimplantation reprogramming processand then to characterize their role, mechanism and regulation. It would be interesting and useful to know the exact mechanisms of the epigenetic control of gene expression. Finding out them we would be able to operate directly genes and engineer cells with desired traits for therapeutic purposes. If we characterize these fundamental processes we could get closer to the understanding of general genome regulation where a network is building up from these basic steps.

O1-053P
Is the 1.9 MDa protein found in the hemolymph of oysters 'cavortin'?

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In 1972 Nielsen & Frieden reported that the hemolymph of oysters contains a high molecular weight carbonic anhydrase (OCA) that differs from vertebrate CAs in many respects as well as size. We began to study this protein by scanning transmission electron microscopy and found its mass to be 1.9 MDa [Hamilton & Wall, unpublished data,

1992]. The STEM image suggests a tripartite arrangement of poorly defined spheres. On SDS-gels the protein has a subunit mass of ca. 22 kDa, but on reduction of S-S bonds, the subunits migrate at ca. 24 kDa, suggesting the presence of internal S-S bonds. Why is the OCA so large, containing as many as 80,000 subunits, and representing >90% of the hemolymph protein? Its dehydratase activity is low compared to the bovine 30 kDa CA, and it has no esterase activity, although an esterase can be separated from it by anion exchange chromatography. In a 2-D gel (SDS after isoelectric focusing) the reduced, carboxymethylated OCA resolves into a spectrum of closely spaced spots with a range of pI values from 5 to 6 at the same size (ca. 24 kDa). When the gel is stained for glycoproteins, the row of spots reacts positively. Deglycosylation reduced the number of spots somewhat but not to a single one. Analysis of eight areas across the spectrum by LC/MS/MS was undertaklen to determine how the oyster hemolymph enzyme is related to known CAs. Only one outlying spot matched bovine carbonic anhydrase. To our surprise, seven others matched the protein that Scotti *et al*. (2001) had found and named 'cavortin' which resembles a mussel hemolymph protein they named 'pernin' which may be related to superoxide dismutase. In 2004 Huvet *et al*. found the gene for cavortin in oysters. Thus the mystery of oyster carbonic anhydrase seems to be solved. We are now examining our high molecular weight protein for SOD activity.

O1-054P
Does initiation of EEC gene expression by neural signals depend on chromatin?

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Denervation experiments in rat intestinal mucosa have verified numerical and spatial mutation of EECs. Signal transduction *via* crypt cell receptors alters the GI-hormone gene activation on the cAMP-dependent activators/coactivators (e.g.,CREB/CBP), whose phosphorylation is the first step..A preinitiation complex (PIC) builds up with activators, general transcription activators (TFIID, IIA, IIB, IIF, IIE, IIJ, IIH) including 14 TAFIIs, and TBP associated with RNA-polymerase II holoenzyme; silent DNA, wrapped with chromatin, induced by deacetylation or methylation, blocks transcription in multiple genes. This block can be removed by ATP-dependent chromatin remodeling factors (e.g., SWI/SNF and NURF), activated by the enzymatic capacity of the DNA activators/coactivators). Many transcription processes switch from activation to repression by intervention of different coactivator domains. e.g.The CREB coactivator CBP can with its "KIX"-domain, activated by CARM-1, block the activation of CREB completely, while CBP with its "bromo"-domain leads to manifold activation of CREB. In CCK transcription the HIH/lZIP coactivator myc/max (a protooncogene) has a positive influence on the activation of DNA, but bHIH/lZIP with mad/max (incl. mSin3A), binding at the same DNA e-box, exerts a negative effect during terminal differentiation of the I-cells. These and many other examples are independent from chromatin transcription initiation. The primary target for the neural signals is the basal DNA transcription apparatus, which with its manifold enzymatic capacity then activates chromatin, facilitating transcription positively or negatively. (Abbreviations: CARM-1 = coactivator associated arginine methyltransferase; bHLH = basal helix-loop-helix/lZIP = leucine zipper transcription factor. Supported by the German Research Council DFG: Ho 936/4/1-4.)

O1-055P
ATCE1, An enigma of a transcription factor within the acrosome

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Atce1 has been affiliated with the CREB3/LZIP subtype of the ATF/CREB transcription factor gene family. However, an in-vitro translated ATCE1 protein binds specifically to NF-κB rather than CRE enhancer element. Atce1 is expressed in a testis specific manner, and within the testis transcripts apprear specifically in late round and in elongating spermatids. ATCE1 could be immunoprecipitated only from testis extracts and from epididymal sperm cells, confirming the gene's tissue and cellular specificity. Immunocytochemical staining revealed an acrosome resembling signal, from the first stages of the development of this organelle in late round spermatids up to mature epididymal cells, where signal is exhibited predominantly on the inner acrosome membrane. This signal persists even after the acrosomal reaction has taken place. In accordance to its acrosomal localization, full length ATCE1 transfected HeLa cells showed ER and golgi localization. Truncation of the C-terminus allowed entrance into the nucleas and transactivation of an NF-κB but not CRE regulated reporter gene. We hypothesize that ATCE1 might be paternaly delivered to the newly formed zygote to serve as a transctription factor involved in zygote gene activation. Microarray analysis of mouse NIH 3T3 fibroblasts transfected with the nuclear active form of ATCE1 revealed upregulation of early embryogenesis related genes, supporting our hypothesis.

O1-056P
Evaluation of glomerular damage in children with high grade of vesicoureteral reflux

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Vesicoureteral reflux (VUR) is the regurgitation of urine through a vesicoureteric junction. Reflux of infected urine especially in high grade of VUR (IV-V) may cause scarring in susceptible kidneys, with the potential for compromise of renal function. The aim of the study was to evaluate the eventual influence of high grade of VUR on glomerular damage using microalbuminuria as a parameter.
Children with VUR grade IV-V (n: 11, aged 1 month-16 years) detected by voiding cystourethrography (VCUG) were investigated. Control group was consisted of 17 healthy children. Microalbuminuria was examined in samples of morning urine specimens using microalbumin/creatinine reagent kit. Serum urea, creatinine levels and creatinine clearance (CCR) were measured as markers of renal function. The mean value of microalbuminuria in children with VUR showed statistically significant increase. We discussed increase of microalbuminuria and decrease of CCR in children with high grade of VUR as a possible consequence of retrograde urine flow (intrarenal reflux), glomerulosclerosis and consecutive hyper filtration.

O1-057P
Effect of polyamines on the expression of the *E. coli* AtoSC two-component system genes and the regulated atoDAEB operon

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Polyamines are indispensable cellular components implicated in many physiological functions. The *E. coli* AtoC protein, also known as antizyme, is a non-competitive inhibitor of ornithine decarboxylase, the key enzyme of polyamine biosynthesis. Antizyme in eukaryotic organisms is induced by polyamines, i.e the end product of the reaction that it inhibits, by a mechanism involving a frame-shift in the translation of its open reading frame and thus it is involved in a feed-back regulatory mechanism of polyamine biosynthesis. The *E. coli* AtoC was also found to be induced by polyamines though the mechanism of the induction is still unknown, Moreover, AtoC has been identified as the response regulator of the AtoS-AtoC two component signal transduction system in *E. coli*. The AtoS-AtoC system regulates the expression of the atoDAEB operon-encoded enzymes that control short-chain fatty acid catabolism upon acetoacetate induction. The sensor kinase AtoS has been reported to localize in the *E. coli* membrane fraction and to autophosphorylate upon sensing high acetoacetate levels. AtoS subsequently phosphorylates the response regulator leading to its activation, which in turn leads to transcriptional regulation of the target genes. Moreover, the DNA sequences that are necessary for AtoC binding and subsequent activation of atoDAEB operon transcription have been mapped.
In the present study we demonstrate results on the role of polyamines, the natural inducers of AtoC, in the transcription of atoS and atoC genes as well as that of atoDAEB operon. Polyamine-mediated induction was measured in an atoSC positive or negative background by cloning the promoter and the beginning of the coding sequence of each gene on a promoterless vector carrying the lacZ gene and assaying for β-galactosidase expression.

O1-058P
Hepatocyte injury by peroxisome proliferators and role of glutathione

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Enhancement of peroxisomal fatty acid β-oxidation (Px-ox) by peroxisome proliferators results in the increase of H_2O_2 production. It has been supposed that H_2O_2 from this enzyme system causes peroxisome proliferator-induced hepatic injury and results in DNA damage, and hence leads to tumour formation in rodent liver. Since an increase rate of Px-ox activity is always higher than that of catalase activity after administration of peroxisome proliferators to rat, an imbalance between H_2O_2 production and its scavenging system is an important reason for the hepatic damage. There have been several subjects to be solved, which are relationships between amount of H_2O_2 production and cell toxicity, or increase of Px-ox activity and catalase activity. In this study, a redox imbalance system was employed to investigate the cellular redox status under peroxisome proliferator treatment in primary culture of rat hepatocytes.
Parenchymal hepatocytes were obtained from male Wistar rats of 7 week-age by collagenase-perfused method. Cells were cultured in DMEM with 5% CS with a density of 2x106 cells/60mm dish. The end of culture period, cells were washed, harvested in a proper solvent according to each assay and homogenated by sonication. Addition of peroxisome proliferator nafenopin to culture medium produced 2.5 times higher Px-ox activity which was determined by labeled acetyl CoA production from 14C-palmitoyl CoA, 1.5 times higher catalase activity by UV assay method decreasing rate at 240 nm, higher lipid peroxidation by TBARS method. Inhibition of catalase by aminotiazol treatment did not affect on Px-ox, and increased both total GSH and lipid peroxidation. On the other hand, cellular GSH deficiency by buthionine sulfoximine treatment markedly reduced NF-induced Px-ox activity and increased lipid peroxidation. Taken together, GSH may contribute on exertion of Px-ox activity, and be enhanced in cellular level under the excess of H_2O_2 to eliminate by catalase, Thus, it is suggested that cellular GSH may have a pivotal role in protection against hepatocyte injury by peroxisome proliferators.

O1-059P
Changes in brain cells chromatin functioning at unilateral gangliosympathectomy

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It is well known that sympathetic nervous system and its peripheral parts, especially upper cervical sympathetic ganglia, have adaptational-trophical action on subcellular structures and cells in whole. In this view the changes in brain cells chromatin phospholipids (PL) and their fatty acid compositions, histones, protein-kinase activity were studied at unilateral gangliosympathectomy (removal of right upper cervical sympathetic ganglia).
Data obtained show significant increase in content of lysophosphatidylcholines, decrease of sphyngomyelins and unsaturated, particularly polyenic fatty acids. These results can be as a cause of inhibition of chromatin matrix activity, which is testified by qualitative and quantitative changes in chromatin histone fractions and with depression of protein phosphorilation as well, which depends on inhibition of cAMP-dependent protein kinase activity. It means that repression of protein synthesis take place during of gangliosympathectomy. These changes directly effect also on physico-chemical properties of chromatin revealed by fluorimetric and infrared spectrophotometric methods. In conclusion, there were shown some peculiarities of upper cervical sympathetic ganglia as peripheral neuroendocrine centers effects on chromatin matrix activity and gene expression in brain cells.

O1-060P
Oxidative stress on the anaerobic sulfate-reducing bacteria *Desulfovibrio vulgaris* Hildenborough: genetics and proteomics analyses

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Even if oxygen is toxic to the anaerobic sulfate reducing bacteria, an increasing number of ecological studies show that they are often found in biotopes where oxygen is present. Oxygen toxicity and the ways by which these anaerobic organisms respond to an oxidatvie stress have to be elucidated. The function of both superoxide reductase and superoxide dismutase has been specified using genetics and biochemistry. However, in order to analysze the global effect of an oxidative stress, the method of two-dimensional protein gel electrophoresis was used to evaluate the changes in levels of proteins following oxygen exposure of *Desulfovibrio vulgaris Hildenborough*. Fifty-seven proteins showed differential expression. Thirty-five decreased and nineteen increased as a specific consequence of oxidative conditions. Proteins that are less abundant under

oxidative stress belong to various functional categories such as nucleic acid and protein biosynthesis, detoxification mechanisms, or cell division. Loss of viability of *D. vulgaris* under oxidative conditions can be directly related to the decrease in the cellular concentrations of these proteins, specifying thus the toxicity of oxygen for the cells. Interestingly, quantitative real-time PCR revealed that genes encoding detoxification enzymes (rubrerythrins, superoxide reductase) are down regulated. Among the proteins that were more abundant under oxygen exposure, a thiol peroxidase, a BCP-like protein, and a putative glutaredoxin were identified. Using RT-PCR, the up-regulation of the genes encoding the thiol-peroxidase and the BCP was demonstrated. That is the first time that these proteins have been shown to be involved in the defense of *D. vulgaris* toward an oxidative stress. Several hypothetical proteins were also detected as differentially expressed. A function in the defense mechanism against an oxidative stress is proposed for these uncharacterised molecules.

O1-061P
"Lonely domain" versus "social domain" – an experimental approach to the evolution of eukaryotic multidomain protens.

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The classical hypothesis of W. Gilbert postulated that the eukaryotic multidomain proteins have evolved by the mechanism known as exon shuffling [see *Science* 1985; **228**: 823-824]. However, the situation seems to be more complex. Two types of protein domains can be distinguished: i) domains that have an evolutionary tendency to be fused to other domains (we call them "social domains"); ii) domains that exist only as single free polypeptides and are not detected in multidomain proteins (these we call "lonely domains"). Here we examined whether "lonely domains" can be successfully fused to other domains i.e. can they be changed to "social domains". We compared two types of proteinase inhibitors: *Cucurbita maxima* trypsin inhibitor (CMTI I; "lonely domain") and the kazal type silk proteinase inhibitor (SPI2; "social domain"). Both studied inhibitors were fused separately to either of two target protein domains: the coat protein (CP) of potato virus Y or β-glucuronidase (GUS). Indeed, when fused to "social domain" (kazal-type inhibitor) specific features and functions of both target proteins were retained. Moreover, the inhibitory activity of the kazal domain was preserved. On the contrary, the "lonely domain" (CMTI I) fused to the CP prevented maintenance of the structural features of CP yet having no impact on the activity of the CMTI I domain. Conversely, the enzymatic activity of GUS fused to CMTI I was retained but the inhibitory activity of CMTI I domain was lost. In summary, experimentally created fusions containing the "social domain" preserved their expected native features and functions while similar fusions to the "lonely domain" were unable to maintain the native functions of fused domains.

O1-062P
Role of PPAR-gamma in activated macrophages

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Macrophages are formed in the tissues from blood monocytes when entering the subendothelial space. From here they migrate to the site of inflammation and become activated by various stimuli (by foreign invaders or by various cytokines). Classical activation leads to the formation of an inflammatory macrophage with a Th1 dominant immune response while alternative activation leads to the appearance of a macrophage with the capacity to restore, immunosuppressive functions and the ability for the elimination of tissue debris along with a Th2 dominant response.
A nuclear receptor, PPAR-gamma has been implicated in various metabolic and immunological functions of the macrophage. We found that PPAR-gamma showed a marked, immediate early induction during monocyte-macrophage transition. We sought to characterize the possible functions of PPAR-gamma in differentially activated macrophages. We used primary human monocytes and mouse peritoneal and bone marrow-derived cells to generate activated macrophages and found that PPAR-gamma was rapidly induced in alternatively activated cells and was down regulated upon classical activation. Importantly, measuring target genes' expression we could show that PPAR-gamma is significantly more active in alternatively activated macrophages than in classically activated ones. By global gene expression experiments we found that not only the degree of inductions but also the number of regulated genes changed in the variously activated cells. By immunohistochemistry we could also show that PPAR-gamma is not equally expressed in all macrophages but mainly in a subset of cells that also expressed markers of alternative activation.
These data suggest that PPAR-gamma expression and activity is restricted to a subpopulation of macrophages that are positive for alternative activation markers. It is very likely that the activation state of macrophages is deterministic for the activity of PPAR-gamma.

O1-063P
Okadaic acid induces phosphorylation and translocation of myosin phosphatase target subunit 1 influencing myosin phosphorylation, stress fiber assembly and cell migration in HepG2 cells

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Myosin phosphatase (MP) is a member of the protein phosphatase-1 (PP1) enzyme family. It consists of a catalytic subunit (PP1c) and a 130/133 kDa myosin binding regulatory subunit (MYPT). It was determined that the myosin phosphatase activity and content of myosin phosphatase target subunit 1 (MYPT1) were correlated in subcellular fractions of human hepatocarcinoma (HepG2) cells. In control cells MYPT1 was localized in the cytoplasm and in the nucleus, as determined by confocal microscopy. Treatment of HepG2 cells with 50 nM okadaic acid (OA), a cell-permeable phosphatase inhibitor, induced several changes: 1) a marked redistribution of MYPT1 to the plasma membrane associated with an increased level of phosphorylation of MYPT1 at Thr695. Both effects showed only a slight influence with the Rho-kinase inhibitor, Y-27632; 2) an increase in phosphorylation of MYPT1 at Thr850 associated with its accumulation in the perinuclear region and nucleus. These effects were markedly reduced by Y-27632; 3) an increased phosphorylation of the 20 kDa myosin II light chain at Ser19 associated with an increased location of myosin II at the cell center. These effects were partially counteracted by Y-27632; 4) an increase in stress fiber formation and a decrease in cell migration, both OA-induced effects were blocked by Y-27632. In HepG2 lysates, OA (5-100 nM) did not affect MP activity but inhibited PP2A activity. These results indicate that OA induces differential phosphorylation and translocation of MYPT1,

dependent on PP2A and, to varying extents, on ROK. These changes are associated with an increased level of myosin II phosphorylation and attenuation of hepatic cell migration.

O1-064P
Mining shotgun sequence data for restriction enzymes and for other toxic proteins

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Whole genome shotgun (WGS) sequencing involves cloning segments of genomic DNA into host cells. One can assume, that cells, transformed by restriction enzyme gene containing segments, would not survive, because the protein expressed from the gene would digest the host's DNA. The absence of restriction enzyme gene containing clones would show up in the in the distribution of sequence reads. When mapped onto the assembled genome sequence, reads, that start outside the boundaries of a restriction enzyme gene, and extend into and over the gene, will be missing. This feature offers a way to identify restriction enzyme genes from shotgun datasets. The new bioinformatics method we present here, is based on this idea. To verify the concept, we have analyzed shotgun data from WGS sequencing of *Haemophilus influenzae*, *Methanococcus jannaschii, Helicobacter pylori, Treponema denticola* and *Methylococcus capsulatus*. We have investigated the statistical properties of clone start distributions in these genomes relative to confirmed and putative restriction enzyme genes. We have compared clone start distributions between several genomes and between different strains. From discrepancies in read overlays (from allele differences) we deduct inactivating, translation blocking, frameshifting mutations. We predict potential new restriction enzyme genes, particularly from *M. capsulatus*.

O1-065P
The essential metal binding protein Mia40 mediates import of proteins into the mitochondrial intermembrane space

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Proteins located in the intermembrane space (IMS) of mitochondria participate in a large number of vital functions in the eukaryotic cell, e.g., protein import, oxidative phosphorylation, metal ion transport and apoptotic signalling. Proteins of one class of IMS proteins are characterised by a low molecular mass. They contain highly conserved cysteine residues and often coordinate metal ions and/or form disulfide bridges. Examples are the members of the small Tim family, such as Tim13 containing a twin Cx3C motif and the copper chaperone Cox17 with a twin Cx9C motif. To reach their functional locations, such proteins after synthesis in the cytosol traverse the outer membrane into the IMS. This process is independent of ATP and the mitochondrial membrane potential. Recent studies suggest that net translocation of these proteins across the outer membrane into the IMS is driven by their folding in the IMS triggered by disulfide bridge formation and/or cofactor insertion. Here we report on the identification and characterization of the essential protein Mia40, the first component of a specific translocation pathway of small IMS proteins that contain cysteine motifs.

O1-066P
Pharmacology and behavior of single carbachol-activated cationic channels in murine gastric myocytes

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In isolated murine ileum single smooth muscle cells, external application of carbachol (CCh) (50 μM) or internal GTPγS (200 μM) in calcium-buffered symmetrical Cs^+ solutions caused an inward current with a peak amplitude of more that 0.5 nA (n=50). The I-V relationship for this current was U-shaped at negative potentials and E_{rev} was close to 0mV. Moreover, CCh caused release of Ca^{2+} from internal stores. An important problem concerns the effect of an influx of calcium on single CCh-activated cationic channel behavior.
Using patch-clamp technique to record from isolated patches in outside-out mode it was found that CCh-activated whole cell current comprised of two types of monovalent selective cation channels with conductances of 17 and 70 pS (n=12). Whole cell current properties are mainly determined by the behavior of the 70 pS channel.
The effect of external (2.5 or 10 mM) and internal (30, 100, 500 nM) Ca^{2+} ion concentrations on single 70 pS channel behavior was investigated. Ca^{2+} added to the bath solution at concentration of 2.5 or 10 mM reduced the cation channel conductance from 70 pS down to 46 pS and 42 pS, respectively, but had no significant impact on P0. Intracellular calcium concentration, $[Ca^{2+}]_i$, was buffered to 30, 100, or 500 nM using a BAPTA-/$CaCl_2$ mixture. The amplitude of the CCh- or GTPgS-evoked whole cell current at 100 nM $[Ca^{2+}]_i$ was several times greater then with 30 or 500 nM $[Ca^{2+}]_i$. Under the conditions of 30 or 500 nM $[Ca^{2+}]_i$ P0 for the 70 pS channel was reduced from 0.2-0.5 to 0.02-0.1. Different intracellular calcium concentrations had no influence upon single channel conductance. External application of quinine (20 μM), La^{3+} (1 mM), SKF96365 (30 μM) caused blocking action on single channel current evoked by CCh or GTPγS in the outside-out patch configuration.

O1-067P
Cloning and characterization of a prolyl 4-hydroxylase from *Chlamydomonas reinhardtii*

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4-Hydroxyproline is found in collagens and collagen-like proteins in animals and in many glycoproteins in plants. It is quite abundant also in green alga *Chlamydomonas reinhardtii*, the cell wall of which is composed entirely from hydroxyproline-rich glycoproteins (HRGPs). Proline hydroxylation in collagens is essential for the formation of stable triple-helical collagen molecules; in plant HRGPs the hydroxyprolines act as O-glycosylation sites. The vertebrate enzyme catalyzing the formation of 4-hydroxyproline, namely collagen prolyl 4-hydroxylase (C-P4H), is an $\alpha_2\beta_2$ tetramer, in which protein disulphide isomerase (PDI) acts as the β subunit. Plant P4Hs, however, are monomeric enzymes, and their catalytic properties differ distinctly from animal P4Hs in that poly(L-proline) is their preferred substrate and not an inhibitor.
A gene encoding a P4H α subunit-like polypeptide was identified from the *C. reinhardtii* genome and cloned. The recombinant Cr-P4H was expressed both in insect cells and in *E. coli* and was found to be an active, monomeric enzyme with a molecular weight of 28 kDa. The Cr-P4H was shown to hydroxylate effectively poly(L-proline) and several synthetic peptides corresponding to proline-rich repeats found in plant HRGPs, and also a collagen-like peptide $(PPG)_{10}$ to some extent. Interestingly, Cr-P4H was found to act preferentially on prolines in X-position in the X-Y-Gly repeats on contrary to the

vertebrate and *A. thaliana* P4Hs, which act solely or preferentially on Y position prolines, respectively. RNAi studies are currently carried out in order to determine the exact role of Cr-P4H in algal cells. The recombinant Cr-P4H has also been crystallized, and determination of its structure is in progress.

O1-068P
Defective embryo and meristems (Dem) directs plant development and interacts with Ran, a highly conserved guanidine nucleotide binding protein

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Coordinated cell proliferation and cell differentiation directs the shape and form of multicellular organisms. The seedling lethal phenotype of the plant defective embryo and meristems (dem) mutant revealed little of the gene's role in plant development. Genetic analysis including a novel approach to somatically mutate or modify expression was used to demonstrate that Dem directs both cell division and cell differentiation in plants. Dem is not homologous to any protein of known biochemical function, however a two-hybrid screen identified Ran (Ras-like nuclear protein) as a potential interaction partner. *E. coli*-expressed proteins confirmed the Dem-Ran interaction *in vitro*. Ran is a guanine nucleotide binding protein that is highly conserved between plants and animals, and in animals has been shown to play key roles in: i) nucleo-cytoplasmic protein transport, ii) export of pre-cursor micro RNA from the nucleus, iii) assembly of the spindle during mitosis, and iv) formation of the nuclear envelop following mitosis. Interestingly, multicellularity evolved independently in plants and animals, and there is no protein homologous to Dem in animals. The nature of the Dem-Ran interaction is being investigated.

O1-069P
siRNA mediated knock-down of the Hedgehog pathway member GLI1 inhibits the proliferation of NT2 neuronal precursor cells

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Hedgehog (Hh) signaling plays a critical role during embryonic development. This pathway thus regulates early development of many organs including limbs, lungs, heart and the central nervous system through multiple effects on progenitor cell populations. In the present study we have investigated the role of Hh signaling on the proliferation of NT2 cells. A teratocarcinoma cell line with stem cell characteristics and the ability to differentiate into neuron-like cells. NT2 cells express most of the known members of the Hh cascade, including GLI1, GLI2, GLI3, PTCH and SMO, but not the ligand sonic hedgehog (SHH). siRNAs directed against GLI1 were designed and investigated for their effects on NT2 cell proliferation. Our results show that Gli1-siRNA is able to reduce GLI1 mRNA expression almost 80% compared to control-siRNA transfected cells and leads to a significant decrease in NT2 proliferation. Interestingly, the Hh antagonist cyclopamine only results in a slight decrease in NT2 proliferation, suggesting that ligand activated Hh signaling may not be of great importance in the regulation of NT2 cell proliferation. This hypothesis is further supported by the lack of SHH expression in NT2 cells. On the contrary, GLI1 expression seems to be involved in growth regulation of NT2 cells, suggesting activation of the Hh pathway downstream of SMO in NT2 cells.

O1-071P
Crystallography suggests that a water molecule provides the sixth ligand to the reduced active iron in superoxide reductase from *Desulfoarculus baarsii*

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Some sulphate-reducing and microaerophilic bacteria rely on the enzyme superoxide reductase (SOR) to eliminate the toxic superoxide anion radical ($O_2^{\bullet-}$). SOR catalyses the one-electron reduction of $O_2^{\bullet-}$ to hydrogen peroxide at a non-heme ferrous iron centre:

$$SOR_{red} + O^{2-} + 2\ H^+ \rightarrow SOR_{ox} + H_2O_2$$

The active iron is coordinated by four histidines and a cysteine in an unusual square-pyramidal geometry. The nature of the sixth ligand varies along the enzyme reaction coordinate. Upon substrate binding to the reduced enzyme, a peroxo intermediate forms, which undergoes two protonation steps to yield hydrogen peroxide. The product is then released from the oxidized enzyme, and it has been shown that, in this oxidized form, Glu47 replaces H_2O_2 and provides the sixth coordination to the active iron. Finally, the enzyme is recycled to its reduced form through the intervention of intracellular reductases.
In the resting, reduced form of SOR, the sixth ligand of the active iron has remained elusive. Crystallographic structures of SOR in this state have not allowed to address this question, because the high accessibility of the active site and its propensity to bind negatively charged molecules have caused non physiological anions to block the site. A vacant coordination has been postulated, but simulations by DFT calculations have also suggested that a water molecule could act as a weak sixth ligand.
Here, we have solved the structure of *Desulfoarculus baarsii* SOR (mutant E47A) to 1.7 Å resolution, in conditions where the reduced active site was left "free". A water molecule was observed bound to the iron, at a distance of 2.9 Å, in line with the DFT calculations. It is possible that this water may serve as a general base during the protonation steps of the peroxo intermediate.

O1-072P
Amino acid preferences for P1 and P4 sites of retroviral proteases in type 1 cleavage sites

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Retroviral proteases have essential role in the life cycle of retroviruses, therefore they may be good therapeautic targets

for treatment of AIDS or leukemia. However, resistant viral strains may quickly developed during the treatment, due to the high mutation rate of retroviruses. Many of the mutations in the protease sequences of drug resistant human immunodeficiency virus (HIV) strains can be found in other retroviral proteases, therefore understanding the specificities of these enzymes may help to design broad-spectrum inhibitors against HIV-1 protease. Previously, substrate specificity for P2 site was studied on a representative set of retroviral proteases, which included at least one member from each of the seven genera of retroviridae [Bagossi *et al*. (2005) *J. Virol*. **79**: 4213-4218]. In the present work we have extended the study of the retroviral protease set of HIV-1, HIV-2, equine infectious anemia virus, avian myeloblastosis virus, Mason-Pfizer monkey virus, mouse mammary tumor virus, Moloney murine leukemia virus, human T-cell leukemia virus type 1, bovine leukemia virus, human foamy virus and walleye dermal sarcoma virus, to study of the amino acid preference for P1 and P4 subsites of a naturally occurring type 1 cleavage site (Val-Ser-Gln-Asn-Tyr«Pro-Ile-Val-Gln) in HIV-1. Molecular models for all studied proteases were built, and they were used to understand the specificity similarities and differences between retroviral proteases. The result showed that retroviral proteases had similar preferences (Phe, Tyr) for P1 site in this sequence context, but more differences were found for P4 site. The proteases were grouped based on their P4 specificities and the classification was correlated well to the philogenetic tree of retroviruses. (This research is supported in part by OTKA T43482, F34479, F35191 and AIDS FIRCA Grant TW01001.)

O1-073P
The membrane microenvironment of P-glycoprotein

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One of the unresolved problems of cancer chemotherapy is the resistance of cancer cells to a broad range of cytotoxic drugs, referred to as multidrug resistance. This phenomenon is frequently associated with the expression of certain ABC transporters including P-glycoprotein (Pgp).
The UIC2 anti-Pgp mAb binds only to 10-30% of cell surface Pgps (pool I), while the rest of the molecules (pool II) can be labeled only in the presence of certain Pgp substrates/modulators. This conformational heterogeneity of Pgps (i.e. the simultaneous presence of UIC2 reactive (pool I) and non-reactive (pool II) Pgps) seems to be accompanied by the topological segregation of the different pools based on confocal microscopy experiments. To further examine the membrane distribution of Pgp molecules, their raft and cytoskeletal associations have been measured by monitoring the detergent resistant fraction of antibody-labeled cell surface Pgps. 50-60% of pool I Pgp molecules are associated with cholesterol rich membrane microdomains (caveolae, rafts), compared to 20-25% in the case of pool II. In NP40 detergent elution experiments we have also detected differences between the degree of cytoskeletal anchorage of the two Pgp populations. In line with the above results, FRAP (Fluorescence Recovery After Photobleaching) experiments have demonstrated that the mobile fraction of Pgp molecules is significantly lower in the pool I population. The associations of Pgp with different membrane microdomains were further examined isolating the detergent resistant membrane fractions on sucrose density gradients. Our results support the idea that Pgp is involved in the transport of lipids (e.g. cholesterol or glycosphingolipids) characteristic of rafts and caveolae, explaining the higher ratio of UIC2 reactive Pgps in these membrane microdomains. (This work was supported by research grants OTKA TO48742 and TO46945. Katalin Goda is recipient of the Bolyai Fellowship from the Hungarian Academy of Sciences.)

O1-074P
What is important in teaching of principal biochemistry

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Biochemistry is a very difficult subject for students, which draws on knowledge from physical and life sciences. Biochemistry has a major impact on toxicology, agriculture, nutrition, medicine, pharmacology, ecology and other fields. In fact, biochemistry became an important component of environmental sciences and due to the fact biochemical education concerns an enormous body of studying population. Teaching of biochemistry is multi-aspect theme as regards scope and tools yet, essential question close each lecturer, sounds what and how to teach?
As a matter of experience at the University of Podlasie we summarized ideas and approaches to put into practice for successful, well received and student-friendly teaching. The list includes the following 'advices': (1) prepare first lecture carefully – make impressive introduction; (2) remark on real biochemistry fundamentals; (3) realize what is particularly difficult for students and explain that in details; (4) set key terms, give summary, submit study questions; (5) organize biochemical round-table discussions; (6) use multimedia for teaching and appealing pictorial fashion, rich in schemes, figures, tables and boxes; (7) analyze and interpret laboratory experiments; (8) integrate theoretical concepts and practical skills; (9) argue ethical aspects of some biochemical topics; (10) make each lecture attractive to students; (11) present up-to-date biochemistry and remember that students should follow up; (12) consider what part of an information is taken-home at once.

O1-075P
New insights into the regulation of cGMP-specific phosphodiesterase 5 (PDE5)

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Nitric oxide and endogenous nitrovasodilators regulate smooth muscle tone by elevation of cGMP and activation of cGMP-dependent protein kinase (PKG). The duration and amplitude of the cGMP signal in smooth muscle is regulated in large part by cGMP-specific cyclic nucleotide phosphodiesterase (PDE5). A principle regulatory mechanism for the control of PDE5 activity is *via* its phosphorylation by PKG. This phosphorylation has been correlated with activation of PDE5 activity. The regulation of PDE5 dephosphorylation is presumed to be as important physiologically as the regulation of PDE5 phosphorylation by PKG. However, the protein phosphatase holoenzyme responsible for PDE5 dephosphorylation has not been unambiguously identified. Previous reports proposed that myosin phosphatase might be responsible for regulating PDE5 activity. The aim of the present study was to test the hypothesis that myosin phosphatase is the holoenzyme that catalyzes the dephosphorylation of PDE5. Our data are inconsistent with this model and suggest a distinct, alternate phosphatase present in smooth muscle can regulate PDE5 dephosphorylation and thus likely contributes to the regulation of smooth muscle tone.

O1-076P
The receptor for pheromone biosynthesis-activating neuropeptide (PBAN): Modeling of ligand-receptor coupling

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Pheromone blends are utilized by a variety of moth species to attract conspecific mates. Moth sex-pheromone biosynthesis follows a circadian cycle with production peaking during peak sexual activity periods of mature adult females. The circadian regulation of pheromone production in moths is due to the release of a neurohormone termed PBAN (Pheromone-Biosynthesis Activating Neuropeptide) to the hemolymph. PBAN is a 33 amino-acid neuropeptide with a minimum active sequence of FXPRLamide in the C-terminal and acts directly on pheromone gland cells of mature adult females through the mediation of calcium and cAMP as second messengers. Recently, we successfully identified a gene encoding a G protein-coupled receptor (GPCR) from pheromone glands of female moths that bears sequence homology to the Neuromedin U GPCR in vertebrates. Moths constitute one of the major groups of pest insects in agriculture and since the emission of sex-pheromone is necessary to attract a mate, the failure to produce and emit pheromone is a potential strategy for manipulating adult moth behavior. In this study, the sequences of the transmembrane helices in the PBAN-Receptor (PBAN-R) were identified using a hidden Markov method and the seven transmembrane helices of the PBAN-R were built, packed and oriented correctly after multiple sequence alignment (MSA) of PBAN-R and several other GPCRs with the X-ray crystal structure of Bovine Rhodopsin. The MSA was enhanced using family specific kink patterns. The resulting PBAN-R structure will be used to identify the binding site of PBAN for the design of potential antagonists intended at interrupting biosynthesis and subsequent release of sex pheromones. (Binational Agricultural Research and Development (BARD) Grants IS-2978-98R and IS-3634-04C supported this study.)

O1-077P
The immunotoxic effects of fumonisin B1 and zearalenone in human lymphocytes

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Fumonisin B1 (FB1) and zearalenone (Zea) are mycotoxins produced by fungi that commonly contaminate dietary staples such as corn, and are therefore of concern to human and animal health. The immunotoxic effects of these toxins on lymphocytes and the implications with regard to immunity was investigated.
Lymphocytes were isolated from the blood of a healthy 21-year-old male according to standard techniques. Serial dilutions of each toxin individually and in combination were used to treat lymphocytes for 12 and 24 hours. The cytotoxicity of the mycotoxins was quantified using the methylthiazol-tetrazolium (MTT) assay, with significance calculated at $p<0.05$.
The MTT assay indicated that FB1 was toxic at low concentrations and mitogenic at high concentrations for 12 and 24 hours while Zea appeared to have stimulatory effects except at high concentrations over 24 hours. Flow cytometry results supported these findings. Combination treatments initially had stimulatory effects which at higher concentrations became cytotoxic. Western blots showed protein-FB1 adducts in the cytoplasmic protein fractions when treated individually and in nuclear protein fractions as well when treated in combination with Zea. Zearalenone formed adducts in both the individual and combination treatments with FB1 in nuclear protein fractions. This study showed that FB1 has cytotoxic and mitogenic effects at the cytoplasmic level while Zea has stimulatory effects at the nuclear level, with FB1 and Zea both acting in the nucleus.

O1-078P
Investigation of FB1-protein conjugation in the lung tissue of swine

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Fumonisin B1 (FB1), a type 2 carcinogen, is the most potent and frequently occurring fumonisin infecting corn staples contaminated with *Fusarium verticillioides*. Fumonisins have been found to be hepatotoxic and carcinogenic in rats and research suggests that FB1 may be part of the aetiology of oesophageal cancer in the South Africa and China, where human consumption of mouldy corn is high. Fumonisins are also known to cause acute toxicity to domestic animals viz. equine leucoencephalomalacia and porcine pulmonary oedema (PPO). The target organs of FB1 in the pig are the lung, liver and pancreas. This study is an investigation into the molecular interactions of FB1 in the pig lung.
Twenty pigs were maintained on diets containing FB1 at concentrations 0, 1, 5 and 10ppm for 5 months. The pigs were then slaughtered and the organs removed. The pig lung (5g) was homogenized in buffer and subjected to differential centrifugation. The homogenate fractions were then separated using SDS-PAGE. The gels were then Western blotted using an anti- FB1 antibody.
The lung protein banding patterns revealed prominent bands in the 5ppm FB1 treated pigs as compared to the other treatments. In addition, the Western Blot analysis demonstrated that the FB1-protein conjugation in the 5ppm treated pigs was the most prominent. This is the first study to show that FB1 can form adducts with cellular protein(s). These results may provide a better understanding into the mechanisms by which FB1 directly and indirectly elicits PPO within the lungs of swine. Further investigations into the identity of the adducts are currently underway. (This was a collaborative study between the Universities of Kaposvar, Hungary and KwaZulu-Natal, South Africa.)

O1-079P
Regulation of cortactin translocation to cell periphery in HepG2 cells upon phorbol ester treatment

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The branching polymerization of cortical actin controlled by the Rac GTP-ase and/or tyrosine phosphorylation is the initial step in the migratory mechanism of most cell types. The F-actin binding protein cortactin binds to cortical actin fibres at sites where branching actin polymerization is nucleated and can be tyrosine-phosphorylated. We found that phorbol myristate acetate (PMA) stimulated the rapid translocation of cortactin to cortical actin when initiated the migration of HepG2 human hepatoma cells, but had no similar effects in COS7 cells. PMA-

treatment increased the activity of Src tyrosine kinase in both the HepG2 and the COS7 cells, but resulted in the Src kinase-dependent tyrosine phosphorylation of cortactin in the HepG2 cells only. However, the PMA-induced translocation of cortactin, which was reduced strongly by the protein kinase C inhibitor bisindolylmaleimide I did not require Src tyosine-kinase activity. In addition, the PMA-induced translocation of cortactin did not require phosphatidylinositol 3-kinase activity, and was not prevented when activated Rac was sequestered by the expression of a dominant negative Pak1 mutant or the Pak1 CRIB domain in HepG2 cells. Our data suggest that in HepG2 cells phorbol ester-activated protein kinase C is able to stimulate branching actin polymerization and subsequent cell migration by a cell specific, Rac-independent and tyrosine kinase-independent mechanism.

O1-080P
Phosphorylation of actin by cAMP-dependent protein kinase

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Functionally irreversible phosphorylation of proteins represents a mechanism for long-term effect of a transient signal. Stabile, translation-dependent phosphorylation of actin has been observed in a leukaemia cell line where cAMP induces apoptosis. In the present study β- and γ-actin mRNA was subjected to *in vitro* translation in the presence of the cAMP-dependent protein kinase (PKA). Isoelectric focusing of the forming product showed that PKA had induced an acidic shift compatible with the incorporation of one phosphate group into either β- and γ-actin. The effect of PKA was strictly co-translational. By replacing Ser-338 with Ala no PKA induced acidic shift was noted, indicating that only Ser-338 was subjected to co-translational phosphorylation of PKA. The co-translational nature of the phosphorylation was ascribed to an altered conformation of full-length actin compared to nascent chain actin. This since native G-actin was not a substrate for PKA, whereas denatured actin and Ser-338 of peptides corresponding to residues 333-347 were readily phosphorylated. The co-translationally phosphorylated actin was resistant to dephosphorylation by phosphatase, whereas denatured phospho-actin and phospho-actin peptides were readily dephosphorylated by phosphatase 2A.
Preliminary studies show that cells overexpressing the Ser-338-Ala mutated actin show a loss of actin stress fibres. We believe that co-translationally phosphorylation may have an importance in the understanding of actin dynamic in cell death and differentiation.

O1-081P
Charge separation and energy transfer in the photosystem ii core complex studied by femtosecond midinfrared spectroscopy

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The core of photosystem II (PSII) contains three major subcomplexes: the reaction center (RC) proteins D1D2-cytb559 that transfer redox potential involved in electron transfer in PSII; and the chlorophyll binding proteins (two core antennas): CP43 and CP47. By using time-resolved Visible pump/MidInfraRed probe spectroscopy in the region between 1600 and 1800 cm^{-1} the absorption changes in the C=O stretches of the chromophore and protein associated with energy and electron transfer in plant PSII core complexes were observed. In this work we have studied PSII cores with open and closed RCs, that were excited at 682 nm with an excitation power of 250 nJ at room temperature. On the basis of global analysis for the particles with open RC the decay components with lifetimes 0.4 ps, 5 ps, 27 ps, 1.6 ns and non-decaying were found. For the particles with closed RC the lifetimes of the decay components are: 4 ps, 60 ps and 3 ns. The 0.4 ps, 5 ps and 4 ps components are related to initial charge separation between the accessory chlorophyll Chl_{D1} and the pheophytin $Pheo_{D1}$ which leads to formation of primary radical pair $Chl_{D1}^{+}Pheo_{D1}^{-}$.The subsequent relaxation of this radical pair leads to formation of secondary radical pair $P_{D1}^{+}Pheo_{D1}^{-}$ on a time scale of 27 ps and 60 ps for open and closed RCs, respectively. For open RCs the 1.6 ns component is due to further electron transfer to QA and represents mixture of two states: $P_{D1}^{+}Pheo_{D1}^{-}$ and $P_{D1}^{+}Q_{A}^{-}$, while the infinite component $P_{D1}^{+}Q_{A}^{-}$ state formation. For closed RCs 3 ns component describes relaxation of $P_{D1}^{+}Pheo_{D1}^{-}$ to the triplet state ^{3}P and to the ground state of P. The same experiments were performed on PSII RC. From the observed spectra we conclude that the first and second radical pairs that are formed in PSII cores are virtually identical to those observed in D1D2 isolated reaction centers of PSII.

O1-083P
Interaction of ATM and chromatin remodelling components may regulate cell cycle checkpoints in response to DNA damage

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The Ataxia Telangiectasia Mutated (ATM) gene encodes a 350 kDa protein whose function is defective in the autosomal recessive disorder Ataxia Telangiectasia (AT). This syndrome is characterised by cerebellar dysfunction, radiosensitivity, chromosomal instability and cancer predisposition in AT patients. In mammalian cells, evidence has been presented that ATM plays a crucial role in the DNA damage response pathway, sensing the presence of double strand breaks (DSB's) and transmitting signals to co-ordinate cell cycle arrest, DNA repair and gene transcription.
The DSB can cause aberrant chromosomal rearrangements, mutations or cell death. The majority of DSB are repaired by either homologous recombination or non homologous end-joining. Defects in either of these two pathways, can compromise genomic integrity increasing the possibility of tumorigenesis. The DNA repair machinery may also require chromatin remodelling activities to repair the DSB. Genetic and biological studies have indicated that remodelling of the chromatin structures surrounding a DSB may facilitate DNA damage signalling and repair.
Recently, it has been proposed that ATM recognition or direct binding of ATM to DSB's is not essential but alterations in chromatin structure triggered by the DNA break could initiate ATM activation. Here we report, for the first time, the identification of an interaction between a chromatin remodelling complex (CRC) protein and ATM. We have demonstrated that CRC is phosphorylated by ATM after DNA damage. Furthermore we show that a reduction in CRC levels slows cell growth and may affect proper cell division. This interac-

tion may reveal a new functional pathway of cell cycle checkpoint regulation through the alteration of chromatin structure in response to DNA damage.

O1-085P
Salt and osmotic stress inducible two-component signal pathways in *Synechocystis* sp. PCC 6803

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Microorganisms respond to hyperosmotic and salt stress *via* changes in the levels of expression of large numbers of genes. Such responses are essential for acclimation to a new environment. To identify factors involved in the perception and transduction of signals caused by hyperosmotic and salt stress, the response of *Synechocystis* sp. PCC 6803 was examined. Synechocystis sp. PCC 6803 a particularly useful microorganism in similar analyses. The knockout libraries of histidine kinases (Hiks) and response regulators (Rres) in *Synechocystis* were screened by DNA microarray and slot-blot hybridization analyses. Several two-component systems designated "Hik-Rre systems" were identified, namely, Hik33-Rre31, Hik34-Rre1, and Hik10-Rre3, as well as Hik16-Hik41-Rre17, as the transducers of hyperosmotic and salt stress. We also identified Hik2-Rre1 as a putative additional two-component system. Each individual two-component system regulated the transcription of a specific group of genes that were responsive to hyperosmotic and salt stress.
We compared the induction factors of the salt stress- and hyperosmotic stress-inducible genes that are located downstream of each system and found that these genes responded to the two kinds of stress to different respective extents. In addition, the Hik33-Rre31 system regulated the expression of genes that were specifically induced by hyperosmotic stress, whereas the system that included Rre1 regulated the expression of one or two genes that were specifically induced either by salt stress or by hyperosmotic stress. Our observations suggest that the perception of salt and hyperosmotic stress by the Hik-Rre systems is complex and that salt stress and hyperosmotic stress are perceived as distinct signals by the Hik-Rre systems.

O1-087P
Challenges in the characterization of lectins in insects and ticks

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Challenges facing scientists in characterizing lectins in insects and ticks are described with possible solutions. Lectins were isolated from different tick species and characterised using hemagglutination assays with various erythrocytes. The role of lectins in various insect species and ticks is given with a view to targeting these molecules as sub unit vaccines for the control of vector-borne diseases. The presentation provides method of isolating these glyco proteins, challenges faced and interventions.

O1-088P
The Effect of PPAR-gamma agonist in HUVEC activation

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Endothelial cell (EC) is located at the interface between blood vessel and tissue. Activation of EC will enhance the expression of many surface molecules and cytokines, lead to leukocytes infiltration to extravasculature tissues and cause inflammatory/immune changes, such as atherosclerosis and graft rejection. Recently, peroxisome proliferator-activated receptor γ (PPARγ), a ligand-dependent transcription factor in nuclear receptor superfamily, has shown a regulatory effect in immune and inflammatory procedures. The aim of the studies is to investigate the PPARγ signaling in endothelial cell activation, by using PPARγ agonist rosiglitazone on human umbilical vein endothelial cell (HUVEC). The confluent HUVECs were treated with PPARγ agonist before activated by TNF-α. Using flow cytometry and Cytometric Bead Array, a group of cell surface molecules and cytokines expression were investigated. The expression of VCAM-1, ICAM-1, MHC-I, IP-10, MCP-1, RANTES and IL-8 by HUVEC were up-regulated after TNF-α activation. Compared with vehicle control, PPARγ agonist rosiglitazone significantly reduced the expression of VCAM-1 (22.5 ± 8.8%, $p<0.05$), MHC-I (14.3 ± 7.0%, $p<0.05$), and IP-10 (51.7 ± 13.2%, $p<0.05$). The expressions of MCP-1 and RANTES were trended to be reduced but not significantly ($p=0.10$ and $p=0.06$ respectively). No changes were observed in IL-8. ICAM-1 was slightly but significantly up-regulated (8.2 ± 4.4%, $p<0.05$). The inhibitive effect of rosiglitazone was, however, not abrogated by PPARg antagonist GW9662, indicating that the inhibitive function is PPARγ-independent. These results clearly revealed an inhibitive effect of PPARγ agonists on HUVEC activation, and provided a potential implication in controlling inflammatory and immune related diseases. (This study is supported by GlaxoSmithKline.)

O1-089P
Selection and identification of RNA molecules bound by RbpA, an RNA-binding protein from the cyanobacterium *Synechococcus* sp PCC 7942

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The genomic SELEX technique was used to investigate the RNA binding selectivity of the RNA-binding protein RbpA from the cyanobacterium *Synechococcus* sp. PCC 7942. A library consisting of 38-88 bp segments of Synechococcus 7942 genomic DNA was constructed by amplification of total genomic DNA and was verified by nested-PCR analysis of a 43 base portion of the rbpB (RNA-binding protein B) gene. Results indicated that the genomic SELEX library should contain 3.38×10^6 different molecules, or one molecule per 1.6 bases in the Synechococcus 7942 genome. For selection, N-terminal histidine-tagged RbpA (H_6RbpA) was used along with RNA transcribed from the library. H_6RbpA-RNA complexes were separated by Ni^{2+}-NTA metal chelate chromatography, the retained RNA molecules were reverse-transcribed and PCR amplified to generate the dsDNA library used in the next round of selection. After 10 rounds of selection, [NaCl] was increased from 75 mM to 150 mM and RNA/protein ratio was decreased from 200:1 to 1:1 and a further 4 rounds were car-

ried out. Following rounds 10 and 14, the amplified dsDNA was cloned and sequenced. Three types of RNA were selected in both rounds: RNA poor in G/U (< 50% G/U), rich in G/U (> 50% G/U), and very rich in G/U (> 90% G/U). Sequence analysis of round 14 G/U rich RNA, identified a putative RbpA consensus binding sequence 5′ UGAAUGGGAGGUG 3′. The six terminal ribonucleotides are similar to a tandemly repeated 5′ GUGGUG 3′ sequence present in the G/U very rich RNA sequences. RbpA has previously been shown to have a strong binding preference for GTP and UTP RNA homopolymers. Comparative sequence analysis identified a number of genes whose expression is potentially regulated by RbpA, including cold-shock inducible genes.

O1-092P
Knowledge based atomic hydration potentials based on a new reference state.

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Adequate modeling of protein folding and function, including protein-ligand and protein-protein interaction depends on availability of contact potentials properly reflecting atom interaction. Despite the significant progress in the field of protein modeling brought by the research effort over the last 30 years, the problem remains unsolved. One relatively successful approach is the so-called knowledge based potentials obtained from a statistical analysis of atom pairwise distance distributions in the known protein structures [1]. We present a new method for constructing knowledge based atom interaction potentials in proteins based on a new reference state. The resulting atomic potentials are highly detailed, continuous, and also very accurate at short distances. We apply this method to produce potentials for protein atoms interaction with water molecules based on statistics for 1312 protein structures with low relative homology [2]. The obtained atom hydration potentials predict experimentally determined hydration sites in proteins using Monte Carlo technique with significantly higher accuracy then other methods including molecular dynamics calculations using conventional potential sets. Application of the atomic hydration potentials to analysis of hydration energy of protein interaction interfaces indicates strong preference for favorable desolvation energy contacts.

References

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O1-093P
Structural rearrangement of ?-haemoglobin induced by ?-haemoglobin stabilising protein (AHSP)

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The α-haemoglobin stabilising protein (AHSP) functions to prevent the toxic effects of α-haemoglobin (αHb) in developing erythrocytes. In its functional form, αHb complexes with βHb to generate haemoglobin tetramers. Free αHb, on the other hand, is unstable and precipitates damaging cellular structures and promoting cell death, as illustrated by the β-thalassaemia diseases in which βHb expression levels are reduced and where toxicity of the resulting excess of αHb directly contributes to anaemia. AHSP binds specifically to αHb and prevents its precipitation. Mouse knock-out studies indicate AHSP is important for protecting cells from αHb toxicity during normal haemoglobin assembly and can act to reduce the severity of the mouse β-thalassaemia phenotype. Of mechanistic importance, binding of AHSP promotes the formation of an unusual form of αHb in which the both the "proximal" and "distal" histidines of the heme pocket bind simultaneously to the heme-iron. This hemichrome form of αHb inhibits formation of harmful reactive oxygen species (ROS) as occurs at the Fe-metal centre of free αHb.
We are investigating structural rearrangements in AHSP and aHb that accompany the formation of αHb hemichrome. Interestingly, AHSP exhibits conformational heterogeneity in solution due to cis-trans isomerisation of the peptide bond N-terminal to proline 30. Upon binding to aHb, this conformational exchange is suppressed such that only a single conformer is detectable in AHSP-αHb complexes. The initial complex between oxygenated αHb and AHSP becomes converted over a period of hours to the hemichrome form. NMR measurements indicate that during this conversion, the only region of AHSP undergoing significant structural change is the loop containing Pro30. However, our data are consistent with the cis-Pro conformer of AHSP in all AHSP-αHb complexes, indicating that cis-trans isomerisation is not the mechanism promoting αHb conversion.

O1-094P
An anti-inflammatory role of Smad6 inhibiting the expressions of NF-κB-mediated pro-inflammatory genes

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Inflammation is progressed by the action of pro-inflammatory cytokines, including IL-1β, TNF-α, IFN-γ and iNOS, and resolved by anti-inflammatory cytokines, such as TGF-β. However, It has not been clearly demonstrated how expressions of pro-inflammatory genes are regulated by TGF-β signaling. We here demonstrate that Smad6 protein, one of inhibitory Smads, inhibits the expressions of NF-κB-mediated pro-inflammatory genes. Expression of Smad6 protein inhibited the expression of NF-κB-mediated reporter in RAW264.7 macrophage cell lines and 239T cells whereas Smad7, another inhibitory Smad, did not. In addition, overexpression of Smad6 by Adenoviral system (Ad-Smad6) decreased the expressions of pro-inflammatory genes such as IL-1beta, TNF-α, IFN-γ, and iNOS, which were induced by IL-1 treatment. These inhibitory effects by Smad6 were due to the decrease of IκBα degradation, and subsequently blocking of nuclear translocation of NFκ-B. This blockade of NF-κB function by Smad6 eventually resulted in the reduction of the cognate DNA binding activity of NF-κB. Furthermore, the inhibition of endogeneous Smad6 by small interfering RNA showed the reduction of anti-inflammatory activities of TGF-beta1 antagonizing the effect of pro-inflammatory cytokines induced by IL-1. Therefore, our present data strongly indicate that Smad6 has inhibitory function for inflammation and reduces expressions of NF-κB-mediated pro-inflammatory genes through blocking the degradation of IκBα and nuclear translocation of NF-κB.

O1-095P
Tracking down expression levels of alternative system pathway factors of diabetic population

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Hyperglycemia associated with diabetes mellitus (DM) alters host immune system resulting in predisposition to infectious

diseases such as head and neck, biliary and urinary tract, skin, soft tissues and boney structure of feet. A high risk of infection in diabetic population may lead to life threatening situations. The early proteins of the alternative complement system pathway comprising of factors P, B and D has been shown to play an important role because they form a membrane attack complex (MAC)-C5-9 which debilitates the target microbes and/or molecules *via* cytotoxic and cytolytic reactions. Patients who are devoid or contain low levels of these proteins may be susceptible to developing chronic infections. We have observed striking differences in partially fractionated circulating serum proteins in diabetic patients (type-II), relative to the non-diabetic controls, through 1-D and 2-D SDS-polyacrylamide gel electrophoresis. Our data, obtained from fifty diabetic patients in the 25-45 year age group who had the disease for less than 5 years, indicated patterns in low and high molecular weight proteins which could be grouped into five different categories with minor differences in their respective levels of protein expression. The most striking difference among them is the low levels and/or the complete absence of the 22 kDa and 52 kDa proteins. On the basis of their apparent molecular weights as well as their low abundance, the 22 kDa and 52 kDa polypeptides might represent the properdin family of proteins (factor P), that has been shown to stabilize the labile C3 convertase (C3bBb). Quantitation of properdin expression levels through ELISA and Western blots is in progress and the obtained data will be presented.

O1-096P
The modular architecture of protein-protein binding interfaces

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Protein-protein interactions are essential for life. Yet, our understanding of the general principles governing binding is not complete. In the present study, we show that the interface between proteins is built in a modular fashion; each module is comprised of a number of closely interacting residues, with few interactions between the modules. The boundaries between modules are defined by clustering contact maps of the interface. We show that mutations in one module do not affect residues located in a neighboring module. As a result, the structural and energetic consequences of the deletion of entire modules are surprisingly small. Contrary, mutations cause complex energetic and structural consequences within their module. Experimentally, this phenomenon is shown on the interaction between TEM1-β-lactamase and BLIP by utilizing multiple-mutant analysis and X-ray crystallography. Replacing an entire module of five interface residues with Ala created a large cavity in the interface, with no effect on the detailed structure of the remaining interface. The modular architecture of binding-sites, which resembles human engineering design, greatly simplifies the design of new protein interactions, and provides a feasible view of how these interactions evolved.

O1-097P
LOV (Light, Oxygen, Voltage) and BLUF (Blue light using Flavin) domain containing proteins: Structure/function relations in two types of flavin-binding blue light photoreceptors

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Blue-light photoreceptor proteins that utilize a flavin as their chromophore are classified into three photoreceptor families, cryptochromes, phototropins (that contain (a) so called LOV domain(s)) and proteins that contain a BLUF domain. LOV domain containing proteins occur in plants, green algae, and bacteria, and they are involved in phototropism, chloroplast movement, and stomatal opening, etc. YtvA from *Bacillus subtilis* has an N-terminal photoactive LOV domain and a C-terminal STAS (Sulphate Transporter and Anti-Sigma factor antagonist) domain. This suggests that this protein can sense light through its N-terminal domain, and transfer the signal to its C-terminal domain, to modulate STAS (de)phosphorylation. YtvA has been described as a regulator of the environmental signaling pathway that activates the general stress transcription factor δB. However, this possible photoreceptor role of YtvA has not (yet) been demonstrated. Experiments performed in the presence of light and dark in an ytvA- and ytvA+ (inducible overexpression) background may provide this evidence.

The BLUF domain containing protein AppA from *Rhodobacter sphaeroides* mediates transcriptional regulation of photosynthesis gene expression by light and oxygen. AppA contains in its C-terminal domain a Cys-rich motif and its N-terminal domain is a BLUF domain that forms a long-lived signaling state upon illumination. To date, AppA is the only known protein that transduces and integrates light and redox signals, even when both domains are expressed separately. Here we will discuss the mechanism of photoactivation of the BLUF domain of AppA and how this signal is transmitted to its C-terminal domain.

O1-099P
Folding and binding integrity of multiple alanine substitution variants of a prototype ligand-binding module from the LDL receptor

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The LA repeats that comprise the ligand-binding domain of the LDL receptor are among the most common autonomously structured extracellular modules found in the non-redundant protein sequence database. Here, we investigate the information content of the amino acid sequence of a typical LA module by constructing sequences with alanine residues at nonconserved positions in the module. Starting with the sequence of the fifth ligand-binding repeat of the LDL receptor (LA5), we created generic LA modules with alanine substitutions of nonconserved residues in only the N-terminal lobe, only the C-terminal lobe, and throughout both lobes of the module. LA variants with alanine residues at as many as 18 of 37 positions fold to a preferred disulfide isomer in the presence of calcium. Indeed, the six cysteines, the C-terminal calcium coordinating residues, two hydrophobic residues involved in packing, two glycines, and five other residues that form side chain-intramodule hydrogen bonds are alone sufficient to specify the fold of an LA module when alanine residues are present at all other positions. The LA variants with multiple alanines in either the N- or C-terminal lobe were then exploited to identify residues of LA5 that contribute to the binding of apoE-containing ligands in LDL receptor-derived "minireceptors", implicating non-conserved residues of the N-terminal lobe of LA5 in recognition of apoE-DMPC.

O1-100P
The effect of desferrioxamine on peroxynitrite-induced oxidative damage in erythrocytes

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The interaction of O^{2-}. and NO gives a powerful oxidizing molecule, peroxynitrite ($ONOO^-$). $ONOO^-$ is a source of hydroxyl radicals and nitrates proteins and oxidizes other macromolecules and is a potent nitrating agent that converts free and protein-bound tyrosine to 3-nitrotyrosine. The determination of 3-nitrotyrosine (3-NT) may reflect the degree of $ONOO^-$ -mediated tissue damage. Therefore 3-NT can be used as an index of $ONOO^-$ involvement. Erythrocytes are particularly susceptible to oxidative damage because of their high content of oxygen. Desferrioxamine, a selective ferric chelating agent and an antioxidant, has a protective effect against free radical-mediated damage. The aim of this study was to investigate the effect of desferrioxamine on peroxynitrite-mediated damage in erythrocytes by measuring the 3-NT level and glutathione peroxidase and Na^+-K^+ ATPase activities *in vitro*.
Materials and Methods: 3-NT levels were determined by HPLC; glutathione peroxidase and Na^+-K^+ ATPase activities were measured by spectrophotometry. The results were presented as mean ± SD, statistical significance of the differences was evaluated using analysis of Mann-Whitney U-test. The difference was considered statistically significant when $p < 0.05$.
Results: In the absence of $ONOO^-$, erythrocyte 3-NT level was not detectable. Peroxynitrite increased the 3-NT level but decreased both enzyme activities. The 3-NT levels in erythrocytes addition of exogenously prepared $ONOO^-$ and then treated with desferrioxamine were 1.36 ± 0.37 and 0.59 ± 0.14 nmol/mg protein, respectively. The difference between was statistically significant.
Conclusion: Desferrioxamine was found to possess an important antioxidant activity as assessed in an *in vitro* system, reducing protein nitration, restoring enzyme activities and maintaining erythrocyte membrane integrity.

O1-101P
Synthetic mimetics of the viral IL-6-binding site of gp130

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The transmembrane glycoprotein gp130 is the shared signal-transducing receptor for a family of four-helix cytokines, of which interleukin-6 (IL-6) is a prototypic member. Viral IL-6, which is encoded by Kaposi's sarcoma associated herpesvirus (KSHV) genome, is a homolog of cellular human IL-6. Viral IL-6 is involved in human diseases, such as Castleman disease, primary effusion lymphoma and multiple myeloma. While human IL-6 requires complexation with the α-receptor in order to interact with gp130, viral IL-6 can bind directly to gp130.
Based on the three-dimensional crystal structure of the gp130-viral IL-6 complex, synthetic mimetics of the discontinious binding site of gp130 for viral IL-6 were designed. We generated, by means of solid phase peptide synthesis in conjunction with chemical ligation, a range of assembled peptides presenting the gp130 fragments that make up its discontinious binding site for viral IL-6. These assembled peptides, along with the constituent individual fragments, were tested in a competitive assay, as well as in a cell proliferation assay, for their ability to compete with gp130 for binding to viral IL-6. These studies indicate that the most contribution to the interaction of the assembled gp130-peptides with viral IL-6 comes from one of the three binding site fragments of gp130. Conformational analysis by CD and 2D-NMR spectroscopy of this fragment (221TWTNPSIKSVIILKY235) provides evidence of an α-helical conformation. The results of the binding studies, the cell proliferation assays, as well as the CD and NMR analyses, will be presented.

O1-102P
Mitochondrial theory of carcinogenesis and of anticancer therapy

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We found that chronic inflammation including viral hepatitis and ulcerative colitis increases the mutation of mitochondrial DNA (mtDNA) in the liver and colon; the frequency of mutations was extremely high with cancer patients but not with control subjects, suggesting that chronic oxidative injury causes mtDNA mutations that enhances free radical generation and nuclear DNA mutations. Because oxidative stress also plays important roles in chemotherapy and its side effects, the relationship between mitochondrial density and sensitivity to anticancer agent. We found that mitochondrial density in rat intestine was the highest at the duodenum, medium at the jejunum and the lowest at the ileum; the sensitivity of epithelial cells to cisplatin was the highest at the duodenum, medium at the jejunum and the lowest at the ileum as judged from the occurrence of apoptosis. Cisplatin increased mitochondrial generation of free radicals in mitochondria-enriched intestinal epithelial IEC-6 cells but not in their α_0 cells, suggesting that mitochondrial density is the key factor for the determinations of the anticancer activity and side effects of cisplatin. We found that renal injury by cisplatin also depends on mitochondrial injury. Either administration of L-carnitine that accumulates in renal proximal tubules or targeting superoxide dismutase to the same compartment markedly inhibited the occurrence of renal injury induced by cisplatin without inhibiting its anticancer action in extra-renal tissues. Based on such observations, we propose a novel strategy for the prevention of cancer and of effective chemotherapy of cancer patients without causing side effects.

O1-103P
NMR detected H-bonds and orientation of helices in calmodulin

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The N- and C-terminal lobes of calmodulin have well defined structures in solution, but N-terminal lobe differs significantly from the X-ray structure in the mutual orientation of the helices [1]. To understand nature of that change we have determined the H-bond $^{h3}J_{NC'}$ couplings at protein backbone. The pronounced change of orientation of helices I and IV appears to be controlled by the H-bonds at the hinges of these helices with the metal binding sites. Specifically, the $^{h3}J_{NC'}$ couplings of the H-bonds connecting the first metal ligation site with the first alpha helix (20→16) and the second metal site with the helix IV (68→64) are much stronger (~0.7 Hz) then expected from the crystal structure (~0.2 Hz). The position of these H-bonds is such that their strengthening could be related to the change of the orientation of the helices. Their prox-

imity to metal sites can explain the effect of metal binding on the interhelical angle [1]. The H-bonds in question were absent from the solution NMR structure that was optimized for orientation of the helices by the residual dipolar couplings (1j7o.pdb) [1]. We refined the NMR structure and where able to satisfy both H-bonds and residual dipolar couplings.

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O1-104P
Free radical production in mitochondria interferes with the metabolism of microglial cells

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Reactive Oxygen Species (ROS) are produced during the normal function of mitochondrial respiratory chain. As a consequence of lesions of respiratory complexes, ROS-production of microglia cells is increased in certain neurodegenerative diseases. The aim of the present study was to clarify whether ROS-production induced by incomplete inhibition of mitochondrial respiratory chain could impair metabolic enzymes in microglia. Dose- and time-dependent inhibition of respiratory complex I and complex III was evoked by rotenone and antimycin in BV-2 microglial cell line. The extent of inhibition on respiration was followed by the measurement of microglial oxygen consumption. The activities of a glycolytic enzyme 3-phosphoglycerinaldehyde-dehydrogenase (PGAD) and two Krebs cycle enzymes; citrate-synthase and aconitase were determined following oxidative stress induced by the inhibitors of respiratory chain. PGAD showed an explicit dose-dependent inactivation with both of the inhibitors. The maximal effect of inactivation was 75±8%. There was no significant difference between the effects of rotenone and antimycin. Inhibition of aconitase was more pronounced with antimycin than with rotenone. The maximal inhibition with high dose of rotenone was around 30%, but with antimycin the extent of aconitase inactivation was 70±9%. In contrast, citrate synthase activity was not influenced by any of the inhibitors. Decreased enzyme activities showed a recovery after 90 minutes of incubation with the respiratory chain inhibitors. We conclude that increased ROS-production as a consequence of respiratory complex deficiencies has effects both on mitochondrial (Krebs cycle) and on cytoplasmic (glycolytic) metabolism and thus may contribute to the pathogenesis of neurodegenerative diseases.

O1-105P
Functional characterization of calcipressin 1 motifs that interact with calcineurin

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One of the major goals of our group is the functional and molecular characterization of calcipressin 1, the protein product of the DSCR1 (Down Syndrome Candidate Region 1) gene. This protein belongs to the calcipressin family. The members of this family are quite conserved from yeast to humans and besides sharing a consensus motif designated FLISPP all of them bind and inhibit the Ser-Thr phosphatase calcineurin (Cn). Cn is the only phosphatase regulated by calcium and calmodulin. This confers Cn a pivotal role in all cell types where calcium acts as a second messenger. Calcipressin 1 (CALP1) is the only calcineurin inhibitor that participates in a negative feedback loop mechanism whose transcription is induced by the activation of Cn. We have described that endogenous CALP1 binds tightly to calcineurin A in human T cells. This interaction takes place through two motifs on CALP1, which act independently. Only one of these two motifs is responsible for the *in vivo* inhibition of calcineurin-mediated NFAT-dependent cytokine gene expression, which is crucial for T cell activation. Nowadays, the inhibition of the calcineurin-NFAT signalling pathway is one of the main challenges in immunosuppressant therapy in order to avoid the severe side effects of the current anti-calcineurinic drugs, CsA and FK506, such as nephrotoxicity, diabetes and cancer, among others. The understanding of the inhibitory mechanism of calcipressin 1 in this pathway would facilitate the development of new anti-calcineurinic drugs for therapeutic use.

O1-106P
Chaperone order of the endoplasmic reticulum disturbed by mutant α1-antitrypsin aggregation

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In eukaryotic cells proteins that destined for secretion enter the endoplasmic reticulum (ER). In this organelle a specific folding machinery of chaperones and chaperone complexes helps proteins to reach their native conformation, and makes posttranslational modifications. In case of mutant and misfolded proteins this set of chaperones and folding enzymes retain the protein-products in the ER by a quality control system, and retrotranslocate them into the cytosol for degradation. In several diseases misfolded proteins cannot be eliminated and accumulate in the endoplasmic reticulum.

Our model was the α1-antitrypsin (AAT) transgenic mouse, which overexpresses the mutant form of human α1-antirypsin. The mutant protein is folding-incompetent, and forms aggregates in the endoplasmic reticulum. This accumulation is responsible for several symptoms; cirrhosis, sterile hepatitis and tumor formation.

We studied endoplasmic reticulum chaperones, associated with the AAT-aggregates. By immunprecipitation we found that protein disulphide isomerase (PDI) strongly binds to the AAT-aggregate, and the connection between several endoplasmic reticulum chaperones, that are involved in the most important complexes is different and impaired (e.g. Grp78, Grp94). Fluorimetric measurements showed that PDI activity is lower in the transgenic samples, which indicates a decrease in the amount of active PDI.

The defect of PDI chaperone activity and the changes in ER chaperone complexes may have an important contribution to the systemic chaperone defects in folding diseases.

O1-107P
Conformational changes of the Na^+/K^+-ATPase large cytoplasmic loop induced by the nucleotide binding

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Na^+/K^+-ATPase is a member of P-type ATPases family that transports sodium and potassium ions across the plasma membrane. Energy for this process is gained from the ATP-hydrolysis. Nucleotide binding to Na^+/K^+-ATPase induces dramatic conformational changes of the enzyme, which are essential for

the translocation of ions to the other site of the membrane. It has been also observed that Na^+/K^+-ATPase associates in the presence of MgATP. We have expressed the isolated H4-H 5 loop of the Na^+/K^+-ATPase alpha -subunit as a (His)6-tag fusion protein in *E. coli*. The wild type sequence of this loop (Leu354-Ile777) contains two native tryptophans W411 and W385. We have observed that the steady-state fluorescent intensity decrease and spectral shift accompanied addition of MgATP. Moreover, these two native tryptophans were subsequently deleted and the double mutant W411F-W385F has been used as a template for other point mutations. We have constructed a set of the single tryptophan mutants performing following point mutations: F404W, F571W, F683W, F426W, L733W, V648W. This set of tryptophans is split over the surface of the loop and can be efficiently used for monitoring of the conformational changes. Conformational changes are reflected as a change in the tryptophan's emission spectrum induced by the nucleotide binding.

O1-108P
Molecular dissection of the key LGS residues involved in the control of glycogen biosynthesis

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Glycogen synthase (GS) catalyses the addition of glucose residues to the non-reducing end of a nascent glycogen chain *via* alpha-1,4-glycosidic bonds, using UDP-glucose (UDP-Glc) as substrate. Two isoforms of mammalian GS have been described; most tissues express the muscle form (MGS), whereas the liver isoenzyme (LGS) appears to be tissue-specific. GS activity is highly regulated *via* phosphorylation and allosteric effectors, mainly glucose 6-phosphate (Glc-6-P).
It is generally accepted that the reaction catalysed by GS is rate-limiting for glycogen synthesis in all organs [1]. The importance of this enzyme in the overall process of glycogen deposition is confirmed by the observation that overexpression of GS in cultured hepatocytes increases glycogen accumulation [2]. This is the consequence of the action of Glc-6-P produced by endogenous Glucokinase (GK). This metabolite causes the allosteric activation of the total amount of LGS through a conformational rearrangement that converts this enzyme into a better substrate for protein phosphatases, which catalyze LGS dephosphorylation, thus leading to an increase in its 'active' form. However, when GK is overexpressed, the increase in Glc-6-P results in a higher degree of activation of the endogenous GS, which also leads to the deposition of larger amounts of glycogen. Finally, when both enzymes are overexpressed, there is a combination of the two effects. Therefore, GK and LGS share the control of hepatic glycogen biosynthesis.
Our aim is to study the importance of 'active' LGS, which appears to be the key factor in glycogen biosynthesis. To this end, we are currently generating adenoviruses of LGS where Ser to Ala mutations have been introduced individually at seven homologous MGS phosphorylation sites. We have also modified the N-terminal sites 2 and 2a together with the C-terminal sites 3a, 3b, 3c, 4, 5. These adenoviruses will provide further insight into the role of GS dephosphorylation in the control of LGS.

References

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O1-109P
Genomic analysis identifies KIF14 as a candidate oncogene and potential prognostic indicator in the 1q minimal region of genomic gain in multiple cancers

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Gain of chromosomal bands 1q31-32 is seen in >50% of retinoblastoma and is also common in other tumors, notably breast and lung. This genomic region is likely to harbor oncogene(s) important in cancer development. To define the minimal 1q region of gain, we used quantitative multiplex PCR. We screened DNA from 55 retinoblastoma tumors and 12 breast cancer cell lines for gain at 14 sequence tagged sites (STSs) spanning 1q25.3 to 1q32.3. SHGC-154194 at 1q32.1 showed the most frequent gain in retinoblastoma tumors (71%), while SHGC-85288 and SHGC-100116 were gained in 7 of 12 breast cancer cell lines. We thus defined a 3.06 Mbp minimal region of 1q genomic gain between SHGC-154194 and next-most commonly gained STSs. This region contains 12 coding genes: C1orf53, LHX9, NEK7, ATP6V1G3, PTPRC, LOC400800, NR5A2, FAM58B, ZNF281, KIF14, DDX59, and CAMSAP1L1. Of these, only KIF14, a mitotic kinesin, showed low expression in normal tissues, and high expression in breast cancer cell lines and retinoblastoma by RT-PCR. Real-time RT-PCR confirmed KIF14 overexpression in 20/22 retinoblastoma tumors and cell lines 100- to 1000-fold higher than in human retina (t-test p=0.00002). Cell lines (n=10) had higher levels than tumors (n=12) (p=0.009). KIF14 was also expressed in 4/4 breast cancer cell lines 31- to 92-fold higher than in normal breast tissue. KIF14 protein was robustly expressed in retinoblastoma tumors and breast cancer cell lines by immunoblot. KIF14 mRNA was also expressed in 5/5 medulloblastoma cell lines 22- to 79-fold higher than in fetal brain, and in 10/22 primary lung tumors 3- to 34-fold higher than in matched normal lung. Patients with lung tumors that overexpress KIF14 showed a trend toward decreased survival. KIF14 is thus a novel candidate oncogene, and has promise both as a prognostic indicator and a therapeutic target.

O1-111P
Effect of heat treatment on receptor binding activity of recombinant human lactoferrin from *Aspergillus awamori*

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Lactoferrin is an iron-binding glycoprotein found in physiological fluids of mammals. It has been suggested to have many biological activities, including protection from pathogens, regulation of iron absorption, immune system modulation and cellular growth promoter activity. Lactoferrin concentration is high in colostrum, particularly in humans. Furthermore, lactoferrin levels are high in human milk throughout lactation, which does not occur in bovine milk. Hence infant formula made from bovine milk is practically devoid of this protein. In several countries, milk formula manufacturers add bovine lactoferrin to infant formulas since human lactoferrin is not available for industrial use. However, whether lactoferrin from different specie exerts its

activity in child is still not well known. Several expression systems including transgenic cows, plants and microorganisms have been used to produce recombinant human lactoferrin (rhLF).
In this work, we studied the receptor binding activity of rhLF from *Aspergillus awamori* (kindly provided by Agennix, USA). Pharmaceutical grade rhLF, which is structurally identical to native human lactoferrin, is manufactured at large scale.
We measured the interaction of iron-saturated and apo-rhLF with receptors of membranes from 15-days differentiated Caco-2. A receptor binding assay, using biotinylated-rhLF, was performed. The binding of biotinylated-rhLF to Caco-2 membranes was specific and saturable. Scatchard plot analysis of the specific binding data revealed a dissociation constant (K_d) of 1.6 x 10^{-7} M and 1.1 x 10^{16} receptors/mg of membrane protein for apo-rhLF; and a K_d of 5.1 x 10^{-7} M and 4.7 x 10^{16} receptors/mg of membrane protein for iron-saturated rhLF.
We also studied the ability of lactoferrin subjected to heat treatment to displace biotinylated-rhLF bound to receptor sites of Caco-2 membranes. Competition curves were made incubating cell membranes with biotinylated-apo-rhLF at a constant concentration in the presence of increasing concentrations of unlabeled apo-rhLF heated at different times. We found a gradual lose of ability to displace biotinylated-apo-rhLF from membrane receptors when the thermal treatment increased.

O1-112P
Effect of human milk on viability of the cell lines MDCK and Caco-2.

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Milk contains a wide variety of substances, some of which have a role as nutrients while others have specific biological functions. These factors may have a first target in the mammary gland influencing its function and growth. Afterwards, once those factors have been released into milk they might exert an effect on development and differentiation of the gastrointestinal tract and of different tissues in the newborn.
The effect of human milk and different fractions of it has been studied on the growth of two cell lines: Caco-2 and MDCK. The effect of skimmed milk, whey and casein fraction from 29 donors at different concentrations has been assayed. The growth rate has been determined by the method of NBB (Naftol Blue-Black) which determines the cellular protein content. Marked differences have been observed in the effect of human milk depending on the milk donor. In the cell line MDCK, skimmed milk (1/10 dilution) and casein (10 mg/ml) from 15 donors reduced cell viability in more than 80%. For Caco-2, cell viability reduction was smaller, between 50 and 80%. It has been also studied the effect of casein (between 1 µg/ml and 10 mg/ml) on cell growth. Inhibition has been observed from concentrations of 5 mg/ml or 10 mg/ml, depending on the cell line and the sample of casein. Chromatin condensation and posterior DNA fragmentation (shown by Hoechst 33258 stain and DNA agarose electrophoresis) are compatible with a cell death through apoptosis mechanisms. The inhibitory effect could be due to the casein itself or to a component associated with it. Different treatments have been made to answer this question. Heat treatment (80ºC, 10 minutes) does not affect to the ability of casein to inhibit cell growth. However, extraction with some polar organic solvents reduce the ability of casein to inhibit cell growth.

O1-113P
Cell-free expression of membrane proteins: A new powerful tool for the structural and functional characterization of prokaryotic and eukaryotic transporters

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Modified cell-free expression systems could provide a new and highly promising technique for the fast and efficient synthesis of functional MPs that have even been completely labeled with stable isotopes. Two different approaches are feasible by using cell-free expression: (a) The production of MPs as precipitates that can easily be resolubilized simply be adding detergent, or (b) the expression of soluble MPs directly in presence of detergents. We have analyzed the optimal conditions for the preparative scale expression of MPs in both ways. A comprehensive variety of detergents has been evaluated for their suitability in the cell-free generation of soluble MPs. We present lists of detergents that are considerably tolerated by the cell-free system and that are highly efficient in the solubilization of even structurally very diverse types of MPs. With our optimized individual cell-free expression system, we demonstrate the high level production (I) of several prokaryotic α-helical transporters, (II) of β-barrel type porins and (III) of eukaryotic G-protein coupled receptors up to amounts of 6 mg in a single milliliter of reaction. The proteins have been analyzed by circular dichroism spectroscopy, by freeze-fracture electron microscopy of reconstituted proteoliposomes and by ligand binding or transport assays. We present evidence that the specific activity of some proteins seems to be strongly correlated with their mode of expression. Amino acid specifically labeled MP samples ready for a structural analysis by NMR spectroscopy can be generated in less than 2 days. We demonstrate the rapid assignment of the transporter TehA by a new combinatorial approach using the cell-free amino acid specific labeling technique.

O1-114P
Unveiling the structural and functional variations in human apolipoprotein E3 and E4

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Emerging evidence has suggested common pathogenic mechanisms in atherosclerosis and Alzheimer's disease. There are three major apolipoprotein E (apoE) isoforms, designated E2 (Cys-112/Cys-158), E3 (Cys-112/Arg-158), and E4 (Arg-112/Arg-158). Whereas apoE3 is considered as a longevity gene, apoE4 is a dual risk factor to atherosclerosis and Alzheimer's disease. We have expressed full-length and N- and C-terminus truncated apoE3 and E4 tailored to eliminate helix- and domain interactions to unveil functional disturbances. C-terminus truncated apoE 1-191 and apoE 1-231 proteins greatly lost the lipid-binding ability as illustrated by the dimyristoylphosphatidylcholine (DMPC) turbidity clearance. The LDL receptor-binding ability, determined by a competition binding of 3H-LDL to LDL-receptor of HepG2 cells, showed that apoE4 proteins with

N- (apoE4 72-299), C-terminus (apoE4 1-231), or complete C-terminus truncation (apoE4 1-191) maintained greater receptor-binding abilities than their apoE3 counterparts. The cholesterol-lowering abilities of apoE-deficient mice were lost significantly for apoE3 72-299 and apoE3 1-231. Therefore, the structural preference of apoE4 to remain functional in solution and possibly *in vivo* may explain the enhanced opportunity of apoE4 to display its pathophysiologic functions. It may also explain in part the higher tendency of apoE4 to aggregate or co-aggregate with Aβ in the pathogenesis of Alzheimer's disease.

O1-115P
The use of multimedia materials in a biochemistry course

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The new communication technologies have laid new in ways for the teaching of Biochemistry. New media that is web compatible material is not only a new way of communication, it is a new form of communication. The web technologies allow not only a textual and graphical format, which is similar to a traditional printed content, but also in a richer format form, which is multimedia. The biochemical material can be taught with the aid of text, graphics, animations, video, interactive animations, etc. which permit an illustration of the dynamic biochemical processes difficult to present/display by more traditional methods. The new media is not a teacher substitute, yet a new teacher's aid, that could be used as much in a traditional class setting with the aid of computers and projector, as well as being accessible to students in the course web side. The aim of the present project is to develop materials for a biochemistry course (first degree level of the Biology major) that could be used for class and web side presentation.
Different commercial programs were used to prepare the materials: Dreamweaver, Fireworks, Flash and Director, Premiere and Acrobat. In addition, Accelrys DS ViewerPro, MDL ISIS/Draw and HyperChem were used to edit, manipulate and export molecular models for class/web site distribution. With the accompanying MDL Chime plug-in in the web page added interactivity was given to these models, reinforcing their usefulness to the student.
As a result, short simulations have been developed to allow the students to enter into the complex phenomena of biochemistry. This formation system has been well accepted by students and student interest in biochemistry has grown in comparison to former years, when only more traditional teaching methods were used.

O1-116P
Preparative parallel protein purification

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The current drug discovery process demands structural information of pharmacologically relevant proteins. In order to increase the output of novel protein structures, improved methods for preparative high throughput (HT) protein purification need to be developed, since most current HT platforms are limited to small-scale and available technology for increasing throughput at larger scales is scarce. We have adapted a ten-channel parallel flash chromatography system for protein purification applications. The system enables us to perform ten different purifications in parallel with individual gradients and UV monitoring. Typical protein purification applications were set up, e.g. ion exchange- and affinity chromatography, and the results from the present system were comparable, with respect to resolution and reproducibility, with those from control experiments on an δKTA purifier system. Finally, lysates from ten *E. coli* cultures expressing different His-tagged proteins were subjected to a three-step parallel purification procedure, combining the above-mentioned procedures. Nine proteins were successfully purified whereas one failed, probably due to lack of expression

O1-117P
14-3-3 protein interacts with nuclear localization sequence of forkhead transcription factor FoxO4.

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The 14-3-3 proteins are a family of regulatory molecules that interact with other proteins in a phosphorylation-dependent manner. 14-3-3 proteins are thought to play a direct role in the regulation of subcellular localization of FoxO forkhead transcription factors. It has been suggested that the interaction with the 14-3-3 protein affects FoxO binding to the target DNA and interferes with the function of nuclear localization sequence (NLS). Masking or obscuring of NLS could inhibit interaction between FoxO factors and nuclear importing machinery and thus shifts the equilibrium of FoxO localization towards the cytoplasm. According to our best knowledge, there is no experimental evidence showing direct interaction between the 14-3-3 protein and FoxO's NLS. Therefore, the main goal of this work was to investigate whether the phosphorylation by protein kinase B, the 14-3-3 protein, and DNA binding affect the structure of FoxO4 NLS. We have used site-directed labeling of FoxO4 NLS with the extrinsic fluorophore 1,5-IAEDANS in conjunction with steady-state and time-resolved fluorescence spectroscopy to study conformational changes of FoxO4 NLS *in vitro*. Our data show that the 14-3-3 protein binding significantly changes the environment around AEDANS-labeled NLS and reduces its flexibility. On the other hand, the phosphorylation itself and the binding of double-stranded DNA have small effect on the structure of this region. Our results also suggest that the DNA-binding domain of FoxO4 remains relatively mobile while bound to the 14-3-3 protein. (Supported by Grant 204/03/0714 of the Grant Agency of the Czech Republic, by Research Project 1K03020 of the Ministry of Education, Youth and Sports of the Czech Republic, and by Research Project AV0Z 50110509.)

O1-118P
Serin protease inhibitors in crustaceans

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Inhibitors from lobster, crab, and three species of shrimp were characterized by test tube and electrophoretic assays. Soluble protein was recovered after acid precipitation and tested for inhibitors on several orthologous proteases. They were able to inhibit subtilisin and cod trypsin, and to a lesser extent, bovine trypsin. They were unable to recognize any other protease, such porcine trypsin, elastase, chymotrypsin, or pronase. A cDNA library of one species of shrimp was screening for kazal inhibitors. Two genes were found, a sequence that codified for an inhibitor with a single domain and a partial sequence with at least two domains. Both inhibitors showed a deduced specificity for elastase.

O1-119P
Proteomic studies of the receptors on porcine tracheal ciliated cells for the attachment of *Mycoplasma hyopneumoniae*

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Mycoplasma hyopneumoniae is a highly prevalent pathogen which colonizes the ciliated epithelial lining of porcine respiratory tract. This association leads to progressive loss of cilia and the development of porcine enzootic pneumonia. Previous studies indicate that several carbohydrates and glycoconjugates on the surface of the ciliary membrane are involved in the attachment of *M. hyopneumoniae*. Using a thin-layer chromatography assay, the glycolipid receptors were further identified as the sulfated glycolipid La, Lb and Lc. However, no protein receptors of the porcine tracheal ciliated cells have been suggested for the attachment of *M. hyopneuminiae* so far.
In the present study, the host-pathogen interactions were examined by a proteomic approach. The total proteins of the porcine tracheal ciliated cells were separated by 2D gel electrophoresis and blotted onto nitrocellulose membrane to react with *M. hyopneumoniae*. Western blot analysis indicated that six groups of porcine proteins were bound with mycoplasmal cells. Mascot search after MALDI-Mass revealed that aconitate hydratase, lamin A, heat shock cognate protein, and carbonyl reductase may be involved in the binding with *M. hyopneuminiae*. The host-pathogen interactions may be not a direct protein to protein interaction, but through the carbohydrate moieties appeared on the glycoproteins.

O1-120P
New proteomics tools for *in vitro* synthesis of recombinant proteins

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The number of protein coding sequences generated by genomics projects has led to an explosion in the number of gene products being investigated. Conventional methods for synthesizing recombinant proteins – cloning sequences into expression plasmids, transforming, selecting, and growing bacteria and subsequent protein purification – are time-consuming and labor-intensive. It was our aim to produce a system that streamlines the protein synthesis process, but that also embraces the entire protein project workflow – from initial small-scale high-throughput screening procedures up to generation of milligram quantities of protein for structural determinations.
We demonstrate that using the EasyXpress™ range of *in vitro* protein synthesis kits, generation of multiple expression constructs by PCR enables expression optimization. By employing large-scale reactions and addition of an affinity tag to the expression construct, sufficient Se-Met or SI-labeled protein for a thorough structural determination can be synthesized and purified to homogeneity in a single working day. We also present new solutions for non-radioactive co-translational protein labeling: a procedure for random labeling allowing extremely sensitive protein detection, and the first commercial system for site-specific Biotin labeling for immobilization and protein-protein interaction analyses. Moreover, we demonstrate synthesis of eukaryotic proteins containing a wide range of posttranslational modifications using *Spodoptera frugiperda* insect cell-based extracts.
Cell-free expression systems are an attractive option for researchers looking for a fast access to their protein of interest, as they do not require specialized equipment, use simple procedures, and they offer the ability to express proteins that may be poorly expressed in *in vivo* systems. Moreover, the synthesis procedure delivers fully functional proteins and is rapid compared to conventional methods.

O1-121P
Runge-Kutta numerical resolution of the Kellershon-Laurent prion

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In this study, numerical resolution of the non linear differential equation system governing the prion kinetic model proposed by Kellershon and Laurent, is made using the forth order Runge-Kutta method, which allow to follow according to the time, the evolution of the four variables ([PrPc], [PrPres], $[PrPres]_2$, $[(PrPc)(PrPres)_2]$) of the kinetic cycle, in ranges of well defined flow entry (production and infection) and kinetic constants. Parametric analysis shows that the variation in the initial concentrations of the four variables has not major influence on the final stationary state, even when the increase of the flow entry (overexpression and infection) induce the pathogenic state. The influence of kinetic constants (conversion of [PrPc], dissociation of $[(PrPc)(PrPres)_2]$, deterioration of [PrPc], association $[(PrPc)(PrPres)_2]$), formation of [PrPres], formation of$[PrPres]_2$ from $[(PrPc)(PrPres)_2]$) do not show any implication in the disease apparition, when kinetic constants (aggregation of [PrPres], and formation of $[PrPres]_2$ from [PrPres]) induce the pathogenic state. The results presented in this work have been computed by program executed with matlab 6.0.1. The calculated numerical values of the four variables are in the range of the actual data for hamster, showing that the linear combination of used parameters is significant.

O1-122P
Detection of DLK1 subforms in GH-secreting pituitary adenomas

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While aiming to characterise the gene expression profiles of pituitary tumors with the latest generation of Affymetrix gene chips©, a molecule being >200x differentially expressed in comparison of two distinct subtypes of this class of tumors became the major subject of our investigations. This molecule – delta-like 1 (DLK1) – is a member of the epidermal growth factor (EGF)-like family of proteins, which are known to regulate cell fate and differentiation decisions during development in many organisms.
In growth hormone (GH)-secreting tumors, a short and probably circulating subform of DLK1 was found to be vastly and very specifically upregulated. Currently, we are interested in the physiological impact of this subform, especially when abundantly expressed. On the other hand, we discovered an uncharacterised splice variant of the DLK1 gene, which we named “secredeltin”. Secredeltin is lacking EGF-like domain

6 and is, compared to the full-length molecule, strongly represented in a subset of neuroendocrine tumors. The abundance of secredeltin vs. full-length DLK1 mRNA is as far as 45x higher in GH-secreting pituitary tumors compared to normal pituitary tissue. Similar results were only obtained in pheochromocytomas. To date, the impact of this finding is not clear, although it may be speculated that DLK1 function varies depending on the ratio of DLK1 full-length vs. secredeltin. Due to its cell differentiation/proliferation regulating nature, effects on tumor growth are conceivable.

O1-123P
Regulation by ethanol of hepatitis B virus replication: Requirement of JAK-STAT signaling

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Excess alcohol intake is another major cause of cirrhosis and hepatocellular carcinoma. In patients infected with hepatitis B virus (HBV), alcohol intake may exacerbate clinical course of acute or chronic HBV infection and make effects on the susceptibility to HBV-related disease, such as cirrhosis and hepatocellular carcinoma. In this study, we examined whether ethanol makes an effect on HBV replication and which signaling pathway is involved in the event. Firstly, increase of HBV transcripts level showed dose- and time- dependency in ethanol-treated HepG2.2.15 cells, which is known to produce hepatitis B virus particles. The viral DNA of HBV was also induced by 200 mM ethanol treatment for 24 hrs in HepG2.2.15 cells. Ethanol exposure induced the phosphorylation of STAT3. And ethanol-induced phosphorylation of STAT3 was decreased by the inhibitor of JAK2 (AG490). Increase of HBV promoter activity by ethanol was abolished by pretreatment with AG490 in HepG2 cells. The HBV transcripts induced by ethanol were perfectly inhibited by AG490, whereas the inhibitor of MEK (PD98059), the inhibitor of JNK (SP600125) and the inhibitor of p38 (SB203580) had no effect. Also ethanol-enhanced HBV DNA synthesis was blocked by pretreatment of AG490. These results suggest that JAK-STAT signaling plays an important in ethanol- enhanced replication of HBV.

O1-124P
Modeling von Willebrand factor catabolism helps to explain the pathogenesis of the Vicenza variant of von Willebrand disease

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Von Willebrand disease (vWD) is the most prevalent bleeding disorder in man. It is caused by quantitative or qualitative deficiency of the von Willebrand factor (vWF), a plasma glycoprotein that plays double role in coagulation: it is an adhesive molecule that connects platelets with subendothelial structures once a vessel wall is damaged, and functions as a carrier molecule of factor VIII in the circulation. VWF is synthesized and stored by the megakaryocytes-platelets and the endothelial cells, and secreted into the plasma in forms of large multimers often weighting more than 10.000 kDa. VWF is cleaved by the enzyme ADAMTS-13, resulting in a 176 kDa and a 140 kDa product at the ends of the cleaved molecules. While the clearance mechanism is currently unknown, estimated half life of vWF is about 12 hours. Since different sizes of the protein are simultaneously present in the plasma, but only the large ones are biologically active, the electrophoretic multimer pattern is characteristic for several types of the disease.

The Vicenza variant of vWD is characterized by low levels of total plasma vWF, presence of ultra large multimers while the smaller multimers are almost absent, which manifests in a mild bleeding tendency in these patients. The pathogenesis of Vicenza vWD is currently unknown.
We developed a mathematical and graphical model that simulates secretion, cleavage and clearance of circulating vWF molecules and graphically presents the resulting electrophoretic pattern. Modeled multimer distributions were compared to those determined experimentally for patient plasma samples. The following initial assumptions were used: (1) secretion occurs at a fixed rate with the initial ultra large multimer distribution seen in platelet alpha granules; (2) cleavage of the multimers occurs with a probability that increases with increasing molecule size; (3) clearance occurs with a time constant determined by the plasma half life and is independent of molecule size.
Decreasing the half life to 2 hours produces a low plasma concentration of vWF in the presence of ultra large multimers typical of vWD Vicenza without change in any other parameters. Our modeling confirms the hypothesis that the phenotype Vicenza variant vWD is a result of increased clearance of the vWF molecules. This model could be a useful tool in analyzing vWF catabolism in a number of clinical situations including other variants of vWD and related disorders such as thrombotic thrombocytopenic purpura.

O1-125P
Study on enzymatic properties of partially reduced human a-thrombin

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Alpha-thrombin, the multifunctional serine proteinase of the blood coagulation cascade, is two-chain enzyme, which in the case of human species consists of the 36-residue A-chain and the 259-residue B-chain. The chains are connected covalently by a disulfide bridge and are not organized in separate domains but form a single contiguous body. The A-chain not involves in substrate or inhibitor binding, and its role is unknown. The B-chain has been shown to be homologous to the catalytic domains of other serine proteinases. We have demonstrated previously that partial reduction of thrombin by dithiothreitol in rather mild conditions without denaturing agents resulted in disruption of S-S-bond only between A- and B-chains of the enzyme and not affected the other 3 internal disulfide bridges, located in the B-chain.
The purpose of our work was further study on enzymatic properties of the partially reduced thrombin and its separated B-chain by using low-molecular-weight synthetic substrate (TAME) or inhibitors (the derivatives of p-tosyl-L-arginine morpholinyl amides) as well as high-molecular-weight natural substrate e.g., fibrinogen. The synthetic compounds have been prepared by well-known procedures of peptide chemistry. The activities of native, chemically modified thrombin and separated B-chain were measured under study-state conditions at pH 8.5. To characterize the catalytic properties of the enzyme chromogenic substrate S-2160 was also used.
The separation of A- and B-chains of the selectively reduced thrombin has been performed by gel filtration. Although solubility of the B-chain after separation is diminished rather sharp, the isolated B-chain retains (according to the tryptophan fluorescence) native conformation. In spite of this its clotting activity reduced 10-fold as compared to the initial thrombin, and the esterolytic or amidolytic activity decreased 3-fold. These data suggest that the presence of the A-chain in molecule of thrombin promote the normal functioning of the enzyme active site.

O1-126P
The trans-activator of HIV-1 transcription reduces fat mass by increasing total energy expenditure in mice and rabbits

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Wasting is a major cause of the morbidity and mortality associated with Acquired Immunodeficiency Syndrome (AIDS). We investigated whether Tat, a protein encoded by the Human Immunodeficiency Virus-1 (HIV-1) genome, is the etiological agent of wasting. Tat was found to have a profound effect in the reduction of adipose tissue mass and adipocyte cell size in rabbits, obese $Lep^{-/-}$ mice, and transgenic mice overexpressing Tat, thus implicating Tat's involvement in wasting. Tat increased thermogenesis, oxygen consumption, fatty acid β-oxidation, and locomotor activity. This suggested that Tat increased the total energy expenditure by burning more fat. Tat upregulated genes essential in lipid catabolism, including beta-ARs and VEGF-D. Tat also increased the expression of downstream genes such as HSL, eNOS, PGC-1alpha, and UCP1, which are important in lipolysis, mitochondrial biogenesis, uncoupling heat generation, and respiration.

O1-127P
Should I bind or should I go? B-boxes as novel regulators for protein-protein interaction.

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MID1, mutated in Opitz BBBG patients, encodes a ubiquitously expressed, microtubule-associated protein. The MID1 protein forms part of the RBCC (N-terminal RING-finger-2 B-boxes-Coiled-Coil domains) protein family, members of which are known to fulfill central functions in development and disease. MID1 binds α4, a regulatory subunit of protein phosphatase 2A (PP2A), and thereby targets microtubule-associated PP2A towards ubiquitin-specific degradation. MID1 mutations frequently cluster in the C-terminus of MID1, disrupting its interaction with the microtubules. As a consequence, PP2A can no longer be ubiquitinated and accumulates in the cytosol.
B-boxes participate in protein-protein interactions, but no specific function has yet been assigned to them. While we previously showed that the B-box1 of MID1 interacts with ?4, we now found that the B-box2 plays an important regulatory role in this interaction. Yeast-based galactosidase assays, as well as immunostaining and immunoprecipitation experiments, showed that the B-box2 significantly affects the strength of the interaction between B-box1 and α4 and, surprisingly, the association of MID1 to microtubules. Furthermore, we noticed that some alternatively spliced MID1 transcripts hold a single nucleotide change (c to t), suggesting that RNA editing occurs. Edited transcripts translate into MID1 proteins carrying a mutation in one of the core histidines of B-box2 which abolishes both binding to α4 and association to the microtubules, inferring a negative regulatory role for this editing event on the MID1 protein function.
In summary, our data define for the first time a clear role for both B-boxes in an RBCC protein and, moreover, suggest a novel pathomechanism for Opitz syndrome patients with N-terminally mutated MID1.

O1-128P
Lps-dependency of plasminogen recognition by the pla surface protease of *Yersinia pestis*

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Pla is an outer membrane protease that belongs to the omptin family of aspartic proteases. It is a critical virulence factor for *Y. pestis*, the causative agent of plague that enables the spread of the bacterium from the subcutaneous site of infection into lymph nodes and circulation. Pla activates plasminogen and inactivates the plasmin inhibitor α2-antiplasmin and thus promotes uncontrolled plasmin proteolysis at the infection site. Lipopolysaccharide (LPS) affects the folding and assembly of several outer membrane proteins, including Pla. LPS-binding proteins share a three-dimensional lipid A-binding motif of 2-4 cationic amino-acids, which bind to phosphate and acyl groups in lipid A and is partially present in the Pla structure as well. The mechanism of Pla-LPS interaction was studied in this work. We have previously shown that Pla requires rough LPS for enzymatic activity but is sterically inhibited by the long O antigen chains present in smooth LPS. Plasminogen activation by Pla-positive *Y. pestis* was more efficient when cells are grown at 37oC then at 25°C or 20°C, the amounts of Pla remained the same. Y. pestis is known to produce temperature-dependent changes in the acylation pattern of its lipid A. We used a set of well-characterized *E. coli* LPS molecules to reconstitute purified His6-Pla suspended in detergent solution, and the effects of the LPS molecules on Pla-mediated plasminogen activation were evaluated by measuring the resulting plasmin activity. Our work suggests that a penta- and tetra-acylated lipid A is preferred over hexa-acylated lipid A and that the 4´- and 1-phosphate groups in lipid A are critical for the interaction. The results indicate that Pla is adjusted to function in *Y. pestis* growing at 37°C.

O1-129P
Regulation of poly(ADP-ribose) polymerase-1 by non-B-DNA structures

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Poly(ADP-ribose) polymerase-1 (PARP-1) participates in DNA cleavage and rejoining-dependent reactions, such as DNA replication, recombination and repair. PARP-1 is also important in transcriptional regulation, although the determinants for its binding to undamaged genomic DNA have not been defined. Previously we have demonstrated that PARP-1 can potentially recognize structures alternative to the usual duplex B-form DNA. Here, we used enzymatic footprinting and atomic force microscopy to study PARP-1 binding to cruciforms and locally unpaired regions. Patterns of DNaseI and P1 nuclease reactivity show that PARP-1 binds near the

stem/loop boundaries of the cruciform hairpins. Thus, PARP-1 differs from other cruciform-binding proteins by binding to hairpin tips rather than to junctions. Using various types of DNA substrates with defined structures, we show that DNA hairpins, cruciforms and stably unpaired regions are all effective activators of PARP-1 automodification and poly(ADP-ribosyl)ation of histone H1. Enzyme kinetics analyses revealed that the structural features of a DNA co-factor are important for PARP-1 catalysis activated by undamaged DNA. The affinities of PARP-1 for DNA co-factors follow the order: cruciform > hairpin > loop. DNA structure also influenced the reaction rate: when a hairpin was substituted with a stably unpaired region, the maximum velocity of reaction was decreased almost two fold. Cumulatively, these data show that functional transactions between PARP-1 and genomic non-B-DNA structures may provide a mechanistical basis for PARP-1 mediated regulation of chromatin structure and transcription. (This work was supported in part by the NCI grant CA74175-07 VS.)

O1-131P
19Ras can regulate p73 by reducing MDM2-p73 interaction.

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p73 has been identified as a structural and functional homologue of the tumor suppressor p53. Notably, the C-terminal region of p73 has a regulatory function through interactions with positive or negative regulator. In this study, we identified p73 binding protein, p19Ras by using the yeast two-hybrid technique. Alternative splicing of H-ras precursor mRNA leads to the two transcripts p19Ras and p21Ras. Ras proteins are known to be small membrane-localized proteins. However, p19Ras proteins are localized in the nucleus in addition to cytosol and plasma membrane. We confirmed that p19Ras binds full-length of p73 both *in vivo* and in vitro. Moreover, this interaction occurs in nucleus. Overexpression of p19Ras lead to increase the transcriptional activity of p73 as well as an apoptosis of p73. This enhancing effect of p19Ras on p73 activity seems to be due to the inhibition of p73-mdm2 interaction. Taken together, our data demonstrate that association of Ras and p73 represents novel pathway in nucleus where Ras signaling affects multiple targets and p19Ras can activate transcriptional activity of p73 by alleviating mdm2-mediated p73 inhibition.

O1-132P
Effect of lipid-based diet on some lipid-metabolising enzymes

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The effect of long-term feeding of lipid-based diet (5% and 10% cholesterol) on some lipid-metabolising enzymes was examined by determining the activities of lecithin: cholesterol acyl transferase (LCAT), lipoprotein lipase (LPL) and triglyceride (TG) lipase. The LCAT activity was found to increase significantly ($P<0.05$) in the cholesterol fed rats when compared to control animals fed a normal diet and was also found to be dose dependent. The former group (cholesterol fed rats) also showed relative siginificant ($P<0.05$) increases in the levels of both cholesterol ester and free cholesterol. Similarly, the levels of TG increased significantly ($P<0.05$) in the cholesterol fed rats. Both LPL and TG lipase activities were also significantly ($P<0.05$) increased in the cholesterol fed rats when compared to the control. The implications of these findings are discussed with respect to atherogenesis, hyperlipoproteinuria and hypertriglyceridemia.

O1-133P
Cytotoxicity of RNases is increased by cationization and counteracted by calcium-activated potassium channels

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Calcium-activated potassium channels are involved in control of cell proliferation and differentiation. Here we have revealed their role in overcoming the RNase-induced cytotoxicity. Toxic effects of Streptomyces aureofaciens RNases Sa, Sa2, Sa3, and of RNase Sa charge reversal mutants on the human embryonic kidney cell lines differing only by the presence of calcium-activated potassium channels were characterized. In contrast to other RNases, a basic variant of RNase Sa and RNase Sa3 exhibits significant cytotoxic activity of the same order of magnitude as onconase. Our data indicate the absence of a correlation between catalytic activity and stability of RNases and cytotoxicity. On the other hand, cationization enhances toxic effect of an RNase indicating the major role of a positive charge. Essentially lower sensitivity to cytotoxic microbial RNases of cells expressing calcium-activated potassium channels was found. These results suggest that cells without the calcium-activated potassium channel activity cannot counteract toxic effect of RNases.

O1-134P
Analysis of partial protein structures in the PDB database

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Knowledge of the three-dimensional structure (3D) of proteins is important in order to understand the function of proteins. X-ray crystallography and NMR spectroscopy are successfully used to determine the 3D structures of proteins. However, many proteins do not crystallize as a complete unit, they need to be fragmented or truncated with the expectation that some of the fragments will be crystallized individually. Indeed a large fraction of PDB entries are partial structures: Currently, PDB includes 12,112 partial structures, of which 10,347 were solved by X-ray crystallography. Therefore, it is important to understand the rules explaining crystallization of proteins. Our present research is an attempt to look at the common features of the solved protein fragments in order to study the factors determining the ability of these fragments to be successfully crystallized. Using a non-redundant set of 1,312 partial structures we conducted a research focusing on three interesting aspects. First, the location of solved fragments within the whole proteins. Second, the distribution of amino acids around the cutting points between the crystallized and the non-crystallized regions. Third, the relationships between known domains and crystallized regions.

O1-135P
Thermostability study of *Camelus bactrianus* heavy chain and VHH antibodies specific for 2-7 EGFR

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Camelids have a unique immune system capable of producing single-domain heavy-chain antibodies. It has been shown that the variable domains of these heavy chain antibodies are functional at or after exposure to high temperatures, in contrast conventional antibodies. In this study the VHH camel antibody (clone ORB1-83) was sub cloned and expressed in *P. pastoris*. ORB1-83 VHH antibody recognized the external domain of the mutant EGF receptor, EGFR VIII. This tumour specific antigen is ligand independent, contains a constitutively active tyrosine kinase domain and has been shown to be present in a number of human malignancies. We reported here that, while expression from *P. pastoris* resulted in a significantly increased level of expression of the anti-EGFR VIII VHH antibodies compared to *E. coli* (in previous study). This antibody selectively bound to the EGFR VIII peptide and react specifically with the immunaffinity purified antigen from non small cell lung cancer. Furthermore thermal denaturation stability and circular dichorism spectra analysis of the heavy chain antibodies that were studied, proved reversibility and the binding activity after heat denaturation were fully retained. Our results indicate that the *P. pastoris* expression system may be useful for the expression of camel single domain antibody and the ability to reversibly melt without aggregation, allow them to regain binding activity after heat denaturation.

O1-137P
Biophysical investigations of site-directed MCP-1 mutants

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MCP-1 is a member of the chemokine family of proinflammatory cytokines. It is a potent chemoattractant for monocytes and T lymphocytes. The migration of leukocytes from the circulation into tissues is mediated by MCP-1 through its interaction with the receptor and co-receptors. The receptor, CCR2, is a specific cell surface, seven transmembrane G-protein-coupled receptor expressed on monocytes, T cells, natural killer cells, basophils and endothelial cells. The activation of the receptor is followed by a firm integrin-mediated adhesion, change of shape and extravasation of the leucocytes to the site of inflammation. The co-receptors are heparin sulphate GAGs which are part of proteoglycans situated on the endothelial cell surface. They capture and present chemokines in order to establish a local concentration gradient.
Site-directed mutagenesis was performed in order to resolve the specific residues involved in receptor binding and in interaction with the co-receptor. MCP-1 variants were cloned, recombinantly expressed in *E. coli* and purified by SP-Sepharose cation exchanger and reversed phase (RP18) HPLC. Fluorescence spectroscopy and far-UV CD spectroscopy are the two biophysical methods used to analyse the affinity of the mutants with respect to GAG ligands and to analyse the structural changes as a result of the introduced mutations in comparison to the wild type chemokine.
Given that the oligomerisation of MCP-1 in relation to its biological activity is so far not completely understood fluorescence anisotropy experiments and chemical cross-linking experiments were carried out in order to better understand the quaternary structure of these proteins. Finally, functional studies such chemotaxis assays showed the chemotactic activity of the wild type and the mutants *in vitro*.

O1-138P
Attachment of NE-4C neuroectodermal stem cells to different surfaces: evaluation of cell-substrate interactions by optical waveguide lightmode spectroscopy (OWLS)

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Cell-adhesion is an active process, which is executed by receptor-ligand like interactions between cell surface adhesion molecules and extracellular matrix molecules of the environment. Initial cell surface reactions may trigger multiple responses, which in turn can result in either spreading or detachment of the cell. The set of adhesion and attachment molecules mediating the adhesive behavior of neural stem cells and the kinetics of their interactions are largely unknown. Optical waveguide lightmode spectroscopic (OWLS) methods allow quantifying the deposition of material in a thin (<150 nm) layer above the solid sensor surface. In terms of cells, it provides data on focal adhesion sites, while the rest of the cellular mass remains out of the field of detection. In our studies, the initial phases of attachment of cloned, homogeneous neural stem cells (NE-4C) were investigated both by OWLS technique, tracing the time dependence of the effective refractive index (N_{eff}), and by time-lapse microscopy, recording cell shape modulations. Bare or amino-functionalised sensor surfaces were coated with different attachment-molecules including poly-L-lysine, laminin, various RGD mimetic peptides, some of those coupled with various backbone proteins. Cell suspensions in protein-free, HEPES-buffered artificial cerebro-spinal fluid (ACSF) were introduced into the measuring cuvette, and the cell-surface interactions were monitored for 80-120 min. For control, the "attachment" of cells fixed with paraformaldehyde were also assayed. OWLS recordings revealed characteristic differences between the behavior on adhesive and non-adhesive surfaces and also between actively attaching and passively sedimenting (fixed) cells. The actively spreading cells were clearly distinguishable from the passively spreading cells by their N_{eff} versus time functions. In active cell attachment, N_{eff} did not change during an initial (12-30 min) period, but increased rapidly thereafter. Deposition of either living cells to non-adhesive surfaces or fixed cells to any surfaces resulted in a monotonically increasing Neff reaching saturation. The results indicate that OWLS techniques allow rapid evaluation of cell-matrix interactions and provide tools to characterise the composition of sets of adhesion molecules on cell surfaces.

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O1-139P
Partial purification of topoisomerase I from mycobacterium phlei.

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DNA topoisomerases control several cellular activities by catalysing the relaxation of superhelices which are produced during the replication of DNA. They are found in all cell types and exist mainly in two forms as type I and type II. In recent years, topoisomerases gained importance as the target of several antibacterial and anticancer drugs. Investigation of the inhibitory action of the candidate substances on topoisomerase activity is widely used in drug development. The purpose of this study is to purify topoisomerase I from *Mycobacterium phlei*, which is closely related to *M. tuberculosis*, but nonpathogenic for humans and investigate some of its molecular and kinetic properties.
Topoisomerase I is partially purified with a yield of 1.7% from *M. phlei*, with a method including homogenization followed by DNase treatment, Sephadex G50 and Heparine Sepharose column chromatography steps. Molecular weight of the partially purified enzyme was estimated to be 125 kDa at SDS polyacrylamide gel electrophoresis. Enzyme activity was found to be stable in the pH range of 6.0-8.5. Mg^{2+}, ATP and spermidine were not required for the activity. Camptotecin, which is an inhibitor of eukaryotic topoisomerase I did not inhibit the enzyme at the concentrations studied. It is suggested that characterization of topoisomerases from mycobacteria will hopefully lead to the development of selective drugs that would contribute to the treatment of tuberculosis which is an important health problem all over the world.

O1-140P
Anti-apoptotic role of C-phycocyanin against oxalate mediated Vero cell fatality

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C-phycocyanin (CP) is one of the major biliprotein pigments found in blue green algae such as *Spirulina* (Arthospira) species and having potential antioxidant and radical scavenging properties.
Materials and Methods: We tested the anti-apoptotic role of CP against oxalate mediated Vero cell fatality. Cell viability was examined by MTT assay. The oxalate mediated reactive oxygen species (ROS) production in the Vero cells was quantified by using 2,7-dichlorofluorescein in a fluorescence method. Further, [^{3}H]-methyl-thymidine was used to measure cell cycle progression and DNA synthesis using scintillation counter. The decrease in cell number and reduced DNA fragmentation were considered to be the cause of anti-apoptosis, effected by CP and this was determined using flow cytometry. Also confocal images were developed for visualizing the percentage of apoptotic and non-apoptotic cells with respect to oxalate and CP.
Results: The study has divulged that CP reduced the apoptotic effect of oxalate in a dose dependent manner by inhibiting the formation of reactive oxygen species caused due to oxalate load. Interestingly pretreatment with CP effectively reduced the incorporation of thymidine into DNA with that of the control and reduced fabulously apoptotic bodies as visualized by propidium iodide, indicating the inhibition of apoptosis induced by oxalate. Flow cytometry studies, using propidium iodide in cells (TritonX100 permeabilized), revealed that oxalate induction after 24 h acts as proliferative signal for Vero cells, which show an increase in S-phase percentage and cells progressed into apoptotic pathway and this has been well controlled during pretreatment with CP.
Conclusion: The study implies that CP can serve as potential antioxidants to prevent the vulnerability of oxalate mediated oxidative stress and Vero cell fatality.

O1-141P
Nitrotyrosination of b-tubulin during DMSO-induced cardiac differentiation in mouse embryonic stem cell line P19CL6

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Nitric oxide (NO) is a precursor of reactive nitrating species, peroxynitrite and nitrogen dioxide, which produce modified proteins containing 3-nitrotyrosine. Although the involvement of NO in cardiac differentiation has been suggested through treatment with exogenous chemical NO donor, little is known about the generation of endogenous NO *via* nitric oxide synthase (NOS) and furthermore, about the target proteins of tyrosine nitration. In this study, we confirmed that NO is endogenously produced *via* activation of NOS and that β-tubulin undergoes tyrosine nitration during the progression of DMSO-induced cardiac differentiation. The peroxynitrite level mesured by Griesse method increased from differentiation day 4 and peaked day 8 and returned to basal level afterward, accompanying with phosphorylation of eNOS at Ser-1179 in the same time frame. However, the induction profile of eNOS a little prolonged (day 4 to day 12). Most importantly, the tyrosine nitration level increased as the DMSO-induced differentiation proceeded. However, the treatment of NO scavenger, L-NAME, prevented the accumulation of DMSO-induced tyrosine nitration. Compared with the NO production period (day 4 to day 12) and continuous increase of tyrosine nitration level over day 12, the nitration reaction seems to be irreversible in this system, resulting in accumulation. The detection of tyrosine nitration was exactly coincided with the cells expressing cardiotroponin I, suggesting strong involvement of tyrosine nitration in cardiomyocyte differentiation. Furthermore, we identified the tyrosine-nitrated protein as β-tubulin by LC-MS/MS, which was further confirmed either by immunoprecipitation with anti-tubulin antibody followed by anti-nitrotyrosine antibody or colocalization of both immunostainings in the cells. Taken together, we for the first time demonstrated that the endogenously produced NO specifically modify β-tubulin during DMSO-induced cardiomyocyte differentiation in mouse embryonic stem cell line P19CL6.

O1-142P
Protein phosphatase 2A-dependent p70 S6 kinase inhibition by troglitazone: A Novel regulatory mechanism of protein synthesis and migration

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Troglitazone, a synthetic peroxisome proliferator-activated receptor γ (PPARγ) ligand, has been implicated in the inhibition of protein synthesis that leads to inhibit proliferation and migration in a variety of cells. Here, we report that troglitazone acutely inhibited protein synthesis by a repression of p70 S6 kinase (p70S6K) activity in bovine aortic endothelial cells (BAEC). Under same condition, however, we found no alterations in activity of mammalian target of rapamycin (mTOR)

and eukaryotic initiation factor 4E-binding protein 1. Furthermore, treatment with rapamycin and troglitazone additively inhibited protein synthesis and p70S6K phosphorylation, suggesting that these inhibitory effects of troglitazone are not mediated by mTOR. Overexpression of wild-type p70S6K gene significantly reversed troglitazone-induced inhibition of protein synthesis, indicating an important role of p70S6K. Okadaic acid, a protein phosphatase 2A (PP2A) inhibitor, restored the troglitazone-induced inhibition of p70S6K activity and protein synthesis. Although troglitazone did not alter total cellular activity of PP2A, it increased the physical association between p70S6K and PP2A, suggesting an underlying molecular mechanism. Okadaic acid also significantly reversed BAEC migration inhibited by troglitazone, providing a clinical relevance. Finally, GW9662, a PPARγ antagonist, did not alter all observed inhibitory effects. Thus, we demonstrate for the first time that troglitazone acutely decreases p70S6K activity through a PP2A-dependent mechanism that is independent of mTOR and PPARγ, leading to inhibition of protein synthesis and BAEC migration. These results may provide novel scientific evidence that troglitazone and its related drugs thiazolidinediones can be therapeutically used in cell growth-related diseases, such as cancer and atherosclerosis.

O1-143P
New design of PCR chamber and film heater in PCR lab-on-a-chip showed a good performance on DNA amplification

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The miniaturization of analytical devices by micromachining will give much impacts on medical and diagnostic field. We have made much efforts on development of PCR lab-on-a-chip (LOC) in which DNA amplification and DNA sensing for genotyping were done. In previous work, we made simple 1 microliter volume of silicon PCR chambers through semi-conductor process and amplified a p53 axon fragment successfully. In this work, we presents new design of 2, 3, 14 microliter volume, 290nm depth of silicon PCR chambers in which many ordered 50nm silicon pillars are formed on the PCR chamber bottom by semi-conductor process. Silicon pillars show good influence on heat transfer from heater to PCR reaction mixture so that we could get better results of DNA amplification and reproducibility. We also made a new design of 12 mm x 15 mm electrical film type of heater which has Cu heat spreading layer to avoid overheating of PCR reaction mixture locally. The full DNA sequences of N-acetyl transferase 2 (NAT2) gene, cancer related gene, were successfully amplified from blood genomic DNA and confirmed by DNA size in gel electrophoresis. NAT2 gene product transfers the acetyl moiety to toxic compounds coming into the cell. Acetylated toxic compounds are thought to be changed their three dimensional structure and lose their toxic activities. Si PCR chip, composed of Si multi-chamber and small electrical heater will be used for SNP genotyping through allele specific PCR and fluorescence detection.

O1-144P
Minimal ATPase subdomain of movement helicases of potex- and hordeiviruses

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Plant viruses MPs encoded with the first gene of the triple gene block (TGBp1) belong to superfamily 1 of NTPase/helicases. In contrast with large DNA NTPase/helicases of SF 1, the TGBp1s represent a minimized version of RNA helicases with only two structural helicase domains (1A and 2A) and minimal spacers between seven conservative motifs. It has been shown that TGBp1s are NTPases, RNA helicases and RNA binding proteins. To find the minimal ATPase domain of TGBp1 proteins a series of deletion and point mutants of two types of TGBp1-potex-like TGBp1 of potato virus X (PVX) (gen. Potexvirus) and hordei-like TGBp1 of poa semilatent virus (PSLV) (gen. Hordeivirus) was constructed. The recombinant His-tagged proteins were expressed in *E. coli* and purified on Ni-NTA agarose column and their activities were analyzed. N-terminal part of PVX TGBp1 containing only three of seven NTPase/helicase motifs demonstrated ATPase and RNA-binding activities similar with that of the full-length PVX TGBp1. PSLV TGBp1 mutant without III to VI motifs and N-extension part also retained ATPase activity and RNA-binding properties. Kinetic parameters (K_m and an apparent V_{max}) were measured. It was shown that K_m of ATP hydrolysis were about 11-16 μM for both full-length proteins and the truncated mutants. However, V_{max} differed about 1.5-2-fold indicating that the mutants hydrolized ATP with higher efficiency. Besides Hill coefficient (a rate of cooperativity of protein-RNA interactions) increased in the case of the truncated mutants. Thus, the N-terminal part of TGBp1 helicase domains of both types of viruses with TGB including three NTPase/helicase motifs - I, Ia and II is important for ATP hydrolysis and cooperative RNA-binding. (This work is supported by RFFI grant No 04-04-49356.)

O1-145P
Overexpression of peptidylarginine deiminase IV enhances apoptosis of haematopoietic cells

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Peptidylarginine deiminases (PADIs) convert peptidylarginine into citrulline *via* post-translational modification. One of the family, PADI IV, plays an important role in immune cell differentiation and cell death. To elucidate the participation of PADI IV in haematopoietic cell death, we examine whether inducible overexpression of PADI IV enhances the apoptotic cell death in human leukemia HL60 cells and human acute T cell leukemia Jurkat cells. PADI IV reduced the viability in a dose- and time-dependent manner of human HL60 cells and Jurkat T cells. The apoptosis-inducing activities were determined by nuclear condensation, DNA fragmentation, sub-G1 appearance, loss of mitochondrial membrane potential ($\Delta\Psi m$) and proteolytic activation of caspase 9 and 3. Besides, PADI IV significantly arrests cells in G1 phase before they entrance into apoptotic cell death. PADI IV increases tumor suppressor p53 and its downstream p21 to control cell cycle. In the detection of protein expressions, all protein levels of cyclin-dependent kinases (CDKs) and cyclins are not reduced except cyclin D by reducible PADI IV. Tumor suppressor p53 also expands its other downstream Bax to induce cytochrome c release from mitochondria. According to these data, we suggest that PADI IV induces apoptosis mainly through cell cycle arrest and mitochondria-mediated pathway. Furthermore, p53 maneuver PADI IV-induced apoptosis by increasing intracellular p21 to control cell cycle and by Bax to decline Bcl-2 function, destroy $\Delta\Psi m$, release cytochrome c to cytoplasm and activate the caspase cascade.

O1-146P
Characterization of inflammatory pain inhibition by leukocyte-derived endomorphins

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Endogenous opioid peptide beta-endorphin (END) can be released from immune cells in response to stress or to corticotropin releasing factor (CRF) inducing antinociception in inflamed tissues. Endomorphins (EM) are recently discovered tetrapeptides with strong and selective mu-opioid receptor agonist activity that have been detected in mammalian immune cells.
The goal of this study was to examine the role of immune cell-derived EM-1 and EM-2 in inflammatory pain in relation to END. To this end, anti-EM and anti-END antibodies were used to characterize immune cell content, *in vitro* opioid release and relative contributions to peripheral intrinsic and CRF-induced antinociception of endomorphins versus beta-endorphin in Wistar rats with Freund's adjuvant-induced hind paw inflammation.
Intracellular EM concentrations of immune cells present in the area of inflammation were 10-times higher than those found in inflamed lymph nodes or white blood cells, resembling the END profile in those cells. Inflammatory cells released endomorphins in response to different secretagogues, particularly ionomycin. Anti-EM-1 and anti-EM-2 antibodies attenuated swim stress- and CRF-induced antinociception in a dose-dependent manner. The decreases elicited by these antibodies were relatively smaller than that produced by anti-END.
These data suggest that immunocytes present in the inflammatory area accumulate endomorphins and can release them in a similar fashion to the classic opioid beta-endorphin, alleviating inflammatory pain with lower potency than the latter.

O1-147P
Neo-synthesis of cardiolipins in extreme halophilic Archaea under osmotic stress

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Halobacterium salinarum and an archaeon *Halorubrum sp*., isolated from the salterns of Margherita di Savoia (Italy), have been selected as representatives of extreme halophilic *Archaea* to analyse the response to hypoosmotic stress at the lipid synthesis level. When these extreme halophilic microorganisms were exposed to low-salt conditions, relevant changes in the cell membrane lipid composition occur.
Hbt. salinarum cells possess a non-abundant phospholipid dimer consisting of a glycolipid sulfo-triglycosyl-diether (S-TGD-1) esterified to the phosphatidic acid (PA), i.e. S-TGD-1-PA, called glycocardiolipin (GlyC). On other hand, GlyC is abundant in the purple membrane (PM), which is typically isolated after cell disruption by osmotic shock. We show that osmotic stress specifically induces GlyC increase at expenses of S-TGD-1.
Analogously, membranes isolated after cell disruption by osmotic shock from the *Halorubrum sp*. archaeon are highly enriched in archaeal bisphosphatidylglycerol (BPG) and reveal the presence of a novel phospholipid. ESI-MS and NMR analyses revealed that this novel lipid has the structure of a sulfo-diglycosyl-diether-phosphatidic acid (S-DGD-5-PA), i.e. a phospholipid dimer analogue to S-TGD-1-PA of *Hbt. salinarum*.
We show that osmotic shock induces a specific increase in the content of archaeal BPG and S-DGD-5-PA in *Halorubrum sp*., at expenses of phosphatidylglycerol and the glycolipid S-DGD-5, respectively. In control experiments no changes in the lipid composition of Halobacterium and *Halorubrum* cells were found after mechanical disruption of the cells by French press. We conclude that osmotic shock stimulates the *de novo* synthesis of phospholipid dimers, i.e. cardiolipin analogues, in extreme halophilic *Archaea*.

O1-148P
The mutation -42 C>G in phospholamban gene promoter found in an apical hypertrophic cardiomyopathy family decreases the transcriptional activity of the gene.

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Phospholamban is an endogenous sarcoplasmic reticulum calcium ATPase inhibitor with a regulatory function on cardiac contraction/relaxation coupling. Mutations in phospolamban gene (PLN) have been associated with primary cardiomyopathies.
Methods: We performed a SSCP mutational screening in the PLN gene in a 186 patients afected with either Hypertrophic Cardiomyopathy (HCM, n=106) or Dilated Cardiomyopathy (DCM, n=80). The study included the genomic sequences of promoter, exon 1 and exon 2 (containing the coding region) of the PLN gene. Those samples with abnormal SSCP electrophoresis pattern were sequenced. In order to study promoter strength we constructed reporter plasmids containing the luciferase gene and transient transfection analysis in C6 and C2C12 cell lines.
Results: A heterozygous single nucleotide transition from C to G in the phospholamban promoter, 42 bp upstream of the transcription start site, was found in a 85 years old patient with late onset of familiar HCM. Her brother had been diagnosed of HCM and died because of a stroke (DNA sample not available). One 55 years old son with apical HCM with mild expression also shows the mutation. This nucleotide change was not found in 100 control subjects. The PLN –42 C>G mutation decreases phospholamban promoter activity a 43% and a 47%, respectively, in C6 and a in C2C12 cell lines.
Conclusion: The mutation PLN –42 C>G decreases the transcriptional activity of PLN promoter: this could disrupt the calcium sarcoplasmic regulation mechanism, and then affect cardiac contraction and could therefore be the cause of apical HCM in this family.

O1-149P
Low-resolution structural elucidation of the major mugwort pollen allergen Art v 1

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The major mugwort pollen allergen termed Art v 1 represents a glycoprotein comprised of two domains. Whereas the globular N-terminal domain resembles defensins the hydroxyproline-rich C-terminal domain was postulated to adopt an extended left-helical structure similar to that of collagen. Thus the shape of Art v 1 was referred to as head-tail structure. Here we present investigations regarding the low-resolution structure of Art v 1 applying small-angle X-ray scattering

(SAXS) experiments and molecular modelling to differently deglycosylated preparations, as well as to the recombinant non-glycosylated and natural forms. The characteristic two-domain structure was reflected in the pair distance distribution functions (PDDFs) of the intact natural and recombinant Art v 1 molecules resulting in a 4 nm wide head and 5 nm long but thinner tail. Accordingly, the size of the globular N-terminal domain was certified by SAXS measurements of the recombinant defensin domain. The influences on molecular shape caused by the different types of glycosylation were also reflected in the PDDFs of the differently deglycosylated preparations of the C-terminal Art v 1 domain. Integrating these low-resolution structural insights into molecular modelling, a new and more detailed three dimensional model of Art v 1 could be created. (This work was supported by the national research network S-8802 and project P15909-N04 of the Austrian research fund FWF.)

O1-150P
The role of glycine residues in the acylphosphatase superfamily

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Some glycine residues are higly conserved within the acylphosphatase superfamily. This conservation could be attributed to the minimal steric hindrance of the glycine side chain that allows the polypeptide backbone to adopt configurations not permitted to any other residues. A new hypothesis to explain this high conservation can be ruled out from the observation that many proteins have a generic tendency to form toxic aggregates for cells. Glycine residues may be conserved to prevent aggregation. To test this hypothesis we substituted in human muscle AcP the six conserved glycine (G15, G19, G37, G45, G53 and G69) with alanine residues, moreover the glycine 45 was mutated also to serine, glutamate and arginine. The conformational stability of all the variants is not significantly lower than that of other variants with substitutions of non-conserved residues. According to previous studies, the enzymatic activity is lost only by two variants (G15A and G69A), important in the catalytic process. The propensity of the variants to aggregate and to form amyloid fibrils was investigated by means of experiments under partially denaturing conditions and under physiological conditions. All variants, with the exception of the G15A variant, show an aggregation rate faster than the wild-type protein in both the conditions. There are no differences between glycine to alanine variants and other variants, suggesting that the inhibition of the aggregation process is not due to the presence of alanine residues. Glycine residues are important, at least in the AcP superfamily, to contrast the aggregation process. This is a new observation that adds to other studies and that underlines the importance of preventing aggregation as a driving force in the evolution process. (This work has been supported by Italian MIUR, Project FIRB RBAU015B47_001.)

O1-151P
The Nek1 kinase has structural and functional similarities to the NIMA kinase

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The *Aspergillus* NIMA serine/threonine kinase plays a pivotal role in controlling entrance into mitosis. A major function attributed to NIMA is the induction of chromatin condensation. We show here that the founder murine NIMA-related kinase, Nek1, is larger than previously reported, and that the full-length protein conserves the structural hallmarks of NIMA. Even though Nek1 bears two classical nuclear localization signals (NLS), the endogenous protein localizes to the cytoplasm. Ectopic overexpression of various Nek1 constructs suggests that the C-terminus of Nek1 bears cytoplasmic localization signal(s). Overexpression of nuclear constructs of Nek1 resulted in abnormal chromatin condensation, with the DNA confined to the periphery of the nucleus. The condensation was not accompanied by up-regulation of mitotic or apoptotic DNA condensation markers. A similar phenotype was observed following NIMA overexpression, strengthening the notion that the mammalian Nek1 kinase has functional homology to NIMA.

O1-153P
Amyloid formation of a protein in the absence of unfolding and destabilisation of the native state

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In 5% (v/v) trifluoroethanol, pH 5.5, 25°C one of the acylphosphatases from D. melanogaster (AcPDro2) forms fibrillar aggregates that bind thoflavin T and Congo red and have an extensive β-sheet structure, as revealed by circular dichroism (CD). Atomic force microscopy indicates that the fibrils and their constituent protofilaments have diameters compatible with those of natural amyloid fibrils. Spectroscopic and biochemical investigation, carried out using near- and far-UV CD, intrinsic and ANS-derived fluorescence, dynamic light scattering and enzymatic activity assays, shows that AcPDro2 has, prior to aggregation, a secondary structure content, packing around aromatic and hydrophobic residues, hydrodynamic diameter and catalytic activity indistinguishable from those of the native protein. The native protein was found to have the same conformational stability under native and aggregating conditions, as determined from urea-induced unfolding. In addition, the kinetic analysis rules out a model in which AcPDro2 is viewed to unfold before self-assembly. Although fully or partially unfolded states have a higher propensity to aggregate, the residual aggregation potential that proteins maintain upon complete folding can be physiologically relevant in the housekeeping of living organisms aimed at the preventing aggregation and be directly involved in the pathogenesis of some protein deposition diseases. (This work has been supported by Italian MIUR, Project FIRB RBAU015B47_001.)

O1-155P
Identification of HGF and hypoxia induced protein-protein interactions within the multidocking site of c-Met by T7 phage display

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Hepatocyte growth factor (HGF) is a pleiotropic factor that plays an important role in complex biological processes such as embryogenesis, tissue regeneration, carcinogenesis and angiogenesis. It promotes these responses through binding to its receptor, a transmembrane tyrosine kinase c-Met. Upon ligand binding, c-Met undergoes autophosphorylation of specific tyrosine residues within the intracellular region. Phosphorylation of Y1349 and Y1356 in a cluster of amino acids in the C-terminus of c-Met activates the multidocking site (Y1349VHVX3Y1356VNV). Hypoxia, which is a reduction in the normal level of tissue oxygen tension, induces a tran-

scription programme that promotes an aggressive tumour phenotype. In a recent study, it is shown this transcription programme is effective over c-Met expression and enhanced HGF-induced phosphorylation of c-Met. It also results in greater HGF-stimulated cell motility, invasion and branching. But the mechanism(s) that leads to these biological events is unclarified. In order to identify novel interaction partners of the mulisubstrate docking site of c-Met in the response to hypoxia, we have applied T7 phage display system with accordance of HGF stimulation and hypoxia treatment. The library variants binding to the multidocking site on c-Met receptor are selected by using peptides FIGEH{pTYR}VHVNA and HVNAT{pTYR}VNVKC, which resemble the c-Met multidocking site as baits. Following Colony-PCR and DNA sequencing of the thirteen positive clones, vector-derived ends were removed and the sequences were analyzed by basic local alignment search tool (BLAST). Several candidate proteins were identified after blast analysis for 'search for short nearly exact matches'. These candidate proteins may be important on HGF/c-Met induced biological responses relevant to tumorigenesis.

O1-156P
Sphingosine kinase 2 phosphorylates FTY720: potential interactions with the CB1 cannabinoid receptor

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The immunosuppressant FTY720 2-amino-2-(2-[4-octylphenyl]ethyl)-1,3-propanediol) prevents transplant graft rejections and inhibits a variety of autoimmune disorders. FTY720, in contrast to other immunosuppressive agents, acts to specifically induce sequestration of lymphocytes without causing cytotoxicity or suppressing growth potential. Thus, FTY720 may provide an advantage over current immunosuppressive therapies. We have previously shown that FTY720, a sphingosine analogue, is phosphorylated by sphingosine kinase type 2 to form a sphingosine-1-phosphate mimetic [Paugh *et al. FEBS Lett* 2003; **554**: 189]. P-FTY720 interacts with four of the five S1P receptors which has important implications for its functions. Because the cannabinoid family of receptors are closely related to the sphingosine-1-phosphate receptors, and FTY720 can cross the blood-brain barrier, we have now examined whether FTY720 also interacts with these receptors. The cannabinoid CB1 receptor is expressed in the brain and some peripheral tissues including testis, urinary bladder, and immune system whereas the cannabinoid CB2 receptor is predominantly expressed in the immune system (spleen, tonsils, immune cells). Here we report that in cells heterologously expressing CB1, both FTY720 and sphingosine compete for binding of [^{3}H]SR141716A, an antagonist that specifically targets CB1 receptors, as well as the agonist CP 55,940. Moreover, both FTY720 and sphingosine inhibited maximal stimulation of [^{35}S]GTPgammaS binding by the high efficacy agonist WIN 55,212-2 in a dose-dependent, competitive manner. In agreement, they also drastically reduced activation of ERK and Akt induced by binding of WIN 55,212-2 to CB1. Our results suggest that CB1 may be a novel target of FTY720 in the central nervous system.

O1-157P
Characterization of the interactions of CMS with the actin cytoskeleton

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CMS (p130Cas ligand with Multiple SH3 domains) is a ubiquitously expressed adapter-type protein, which was cloned in a yeast two-hybrid screen, based on its direct interaction with the focal adhesion protein p130Cas. A role for CMS in modulating general architecture has been supported by our finding that it co-localizes with F-actin fibers in membrane ruffles. Here we present evidence that CMS directly interacts with the actin cytoskeleton. Interestingly, by transiently over expressing a series of CMS deletion constructs in Cos7 cells, we noticed changes in the actin cytoskeleton, such as reduced stress fiber formation, and accumulation of perinuclear F-actin. The CMS construct, lacking the coiled-coil domain showed the most profound cytoskeletal collapse. Furthermore, adhesion assays with podocytes expressing CMS deletion constructs indicated a decreased adhesion capacity of the cells expressing a CMS mutant lacking the coiled-coil domain. Using *in vivo* F-actin pelleting assays we found that the actin binding properties of CMS are located in the carboxy-terminal half of CMS, and that the three SH3 domains are dispensable for its association with filamentous actin. To further characterize the CMS F-actin interaction, a series of GST tagged carboxy-terminal CMS constructs were generated, and utilized in *in vitro* pull-down experiments. These experiments revealed that the entire carboxy-terminal half of CMS is necessary for efficient binding.

O1-158P
ABCB6 overexpression is associated with resistance to arsenite, cisplatin, and antimony

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During chemotherapy, cancer cells develop resistance not only to drugs of treatment, but also to structurally and chemically unrelated compounds. This multidrug resistance (MDR) results, in part, because of an energy-dependent drug extrusion mediated by continued expression, or upregulation, of ATP binding cassette (ABC) proteins, including P-gp (ABCB1), MRP1 (ABCC1), and ABCG2. Our data indicate that increased expression of ABCB6 occurs in cells selected for arsenite resistance. Parental KB-3-1 cells were subjected to increasing concentrations of arsenite to generate arsenite-resistant (KAS) cells. KAS cells were 22-fold more resistant to arsenite, and cross-resistant to cisplatin (17-fold) and antimony (11-fold). Microarray and RT-PCR revealed that the ABCB6 mRNA level was 2.5-times higher in KAS cells, and Western blot analysis determined ABCB6 protein expression increased 2.6-fold. In contrast to the other MDR-conferring ABC transporters, ABCB6 expression was reported to occur in mitochondrial membranes, not the plasma membrane, indicating that if it confers MDR, the mechanism might differ from other ABC transporters. To study the role of ABCB6 in heavy metal resistance, KB-3-1 cells were stably transfected with the ABCB6 gene (KB-B6) or empty vector (KB-CV). ABCB6 protein expression in KB-B6 was 16-fold higher than KB-CV and KB3.1 cells. Confocal microscopy and differential centrifugation indicated ABCB6 localizes to both the plasma membrane and the mitochondria, indicating that ABCB6 could potentially function as an efflux pump. We are currently investigating the role of ABCB6 in conferring resistance to arsenite and cisplatin.

O1-159P
Preliminary investigations into the origins of islet amyloid stability

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The aggregation of normally soluble proteins into amyloid fibrils is a unifying event in over 20 human diseases including Alzheimer's, Parkinsons, Creutzfeld-Jakob disease, and type II diabetes. Amyloid fibers display an unusual degree of stability, as evidenced by their resistance to proteolysis and harsh conditions. A better understanding of the source of amyloid stability would provide a framework for designing more effective therapeutic strategies. The aim of this work is to examine the energetic and structural features that contribute to the stability of amyloid fibers formed from short peptide precursors, such as islet amyloid polypeptide (IAPP), the amyloid precursor implicated in type II diabetes. We have employed isothermal titration calorimetry, quantitative mass spectrometry, immunodetection, and light scattering to perform thermodynamic and kinetic measurements of the IAPP fibrillization reaction. Preliminary results regarding the residual monomer concentration, enthalpy of fibrillization, and kinetic stability of IAPP fibers will be presented. This work will provide insight into the driving force of amyloid fiber formation.

O1-160P
The Role of JLP in G?13-mediated JNK signaling

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Scaffolding proteins play a critical role in conferring specificity and fidelity to signaling pathways. The JNK-interacting leucine zipper protein (JLP) has been identified as a scaffolding protein involved in linking components of the JNK signaling module. Gα12 and Gα13, the α-subunits of the heterotrimeric G proteins G12 and G13, stimulate the JNK-module in diverse cell types. Here, we report that Gα12/13 physically interacts with JLP and this interaction enhances G?13-mediated JNK-activation. We also demonstrate endogenous interaction between JLP and Gα13 in MCF7 cells. JLP interaction is specific to G12 family of a-subunits *via* its C-terminal domain (termed GID-JLP), spanning amino acids 1165–1307, and this interaction is more pronounced with the mutationally or functionally activated form of Gα13 as compared to the wild-type Gα13. The presence of a ternary complex consisting of Gα13, JLP, and JNK suggests a role for JLP in tethering Gα13 to the signaling components involved in JNK-activation. Co-expression of GID-JLP disrupts ternary complex formation in addition to attenuating Gα13-stimulated JNK-activity. We also show that the disruption of Gα13-JLP interaction using the GID-JLP attenuates the transforming ability of Gα13, thereby suggesting the potential role this protein-protein interaction on Gα12/13 mediated transforming pathways (Supported by NIH Grant GM49897.)

O1-161P
The role of the protein kinase C (PKC) family in glucocorticoid-induced apoptosis of human leukemic cells

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Glucocorticoids (GC's) are among the most important clinical agents for the treatment of human leukemias. Cells exist, however, that have a functional glucocorticoid (GC) receptor, but are resistance to GC-induced apoptosis—suggesting that major signal transduction pathways (ie PKC family) may influence cellular response to GC. The Protein Kinase C (PKC) signal transduction family has been directly implicated in glucocorticoid-induced apoptosis of mouse thymocytes (with specific members being necessary to GC apoptosis) – however to date this work has not been translated to human lymphoid leukemias. To clarify the role of this family in the human acute lymphoblastic leukemic CEM cell line, we examined the expression, regulation, and activation of the PKC family after treatment dexamethasone (DEX).
Of the 12 known PKC isoforms, 5 were detected at the basal level, and furthermore that specific members (PKC iota) was up-regulated during resistance, while PKC theta was up-regulated during apoptosis. Selectively inhibiting the entire PKC family caused increased cellular susceptibility to GC-induced apoptosis. Selectively targeting specific isoforms (PKC iota) caused resistant cells to become sensitive to apoptosis. Using immunoflouresence imaging, we showed the selective translocation of PKC's iota, theta, and alpha after treatment with glucocorticoids. In addition, we showed specific phosphorylation of the isoforms iota, theta, and alpha in cells undergoing DEX-induced apoptosis. Taken together, these studies show that the PKC family is directly involved in glucocorticoid-induced apoptosis, and that the selective regulation of specific members may explain why some cells become resistant to clinical treatment, while others continue to be sensitive.

O1-162P
Glucocorticoids activate p38 MAP kinase in leukemic cells resulting in apoptosis

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Glucocorticoids induce apoptosis in lymphoid cells through activation of the glucocorticoid receptor with subsequent activation of a complex network of cellular mechanisms, including signal transduction. The p38, a mitogen-activated protein kinase (MAPK), is a highly conserved phosphoprotein reported to be involved in many cellular processes, especially those initiated by cellular "stresses". Activation of p38 MAPK takes place upon phosphorylation of residues threonine 180 and tyrosine 182. Activated p38 MAPK phosphorylates specific down-stream targets. We have recently evaluated the role of p38 MAPK in lymphoid cell apoptosis upon treatment with the synthetic glucocorticoids dexamethasone (Dex) or the 40 times more potent deacylcortivazol (DAC). We show that Dex and DAC stimulate p38 MAPK phosphorylation and increase mRNA of MAP kinase kinase 3 (MKK3), a specific immediate upstream activator of p38 MAPK. Enzymatic assays confirmed elevated activity of p38 MAPK. Pharmacological inhibition of p38 MAPK activity was protective against glucocorticoid-driven apoptosis in human (CEM) and mouse (S49.1) lymphoid cells. To further evaluate the p38 MAPK pathway in response to glucocorticoids we performed analysis using Affymetrix microarrays, real-time RT-PCR, and genetic manipulations by overexpression of MKK3. Microarray experiments of DAC treated glucocorticoid sensitive clone CEM-C7-14 revealed mRNA induction of MKK3 and p38 MAPK. Real-time RT-PCR of glucocorticoid treated CEM-C7-14 cells indicated induction of MKK3 by 8 hours with DAC showing higher levels than Dex. Overexpression of MKK3 was able to evoke glucocorticoid-independent apoptosis in

Dex-resistant CEM-C1-15. Our results clearly demonstrate a role for p38 MAPK signaling in the pathway of glucocorticoid-induced apoptosis of lymphoid cells.

O1-163P
Cryogenic optical waveguide spectroscopy and stark spectroscopy of semiquinone FAD in DNA photolyase

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DNA photolyase is a light-driven flavoprotein that repairs cyclobutylpyrimidine dimers (CPD) in UV-damaged DNA *via* an ultrafast photoinduced electron transfer reaction from the fully-reduced anionic flavin adenine dinucleotide (FADH-) cofactor to the CPD. Using the basic electronic properties obtained from Stark spectroscopy studies, transient absorption experiments have indicated that the electric dipole moment of the CPD induces an electrochromic shift in the electronic transition energies of the oxidized FAD in DNA photolyase. These results suggest that the substrate electric field plays a critical role in the electron transfer process. A similar electrochromic shift has been reported for the energy levels of the semiquinone FAD in DNA photolyase ("Semiquinone PL") upon CPD binding. In an effort to provide further insight into this result, Stark spectroscopy has been employed to study the change in the electronic structure of the electronic states of the semiquinone FAD in DNA photolyase. Quantitative analysis of the Stark spectra requires very-high-quality low-temperature absorption spectra of DNA photolyase solutions. A cryogenic optical waveguide spectrometer (COWS) for dilute biological samples is described. This spectrometer has high reproducibility and affords detection of nanograms of flavoproteins. Additionally, the spectrometer requires several hundredfold less protein than standard low-temperature techniques for the same sensitivity.

O1-164P
Accumulation of large conductance Ca^{2+}-activated K^+ (MaxiK) channels into the sarcoplasmic reticulum in mouse myometrium during late pregnancy

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We have previously demonstrated an overall increase in MaxiK mRNA and protein levels from late-pregnant (LP) mice myometrium, while channel current density and surface expression is diminished as compared to non-pregnant (NP) mice. These observations indicate that although there is an overall increase in MaxiK mRNA and protein levels, the observed reduction in surface expression is due to an altered trafficking of the channels to the cell surface that results in accumulation at perinuclear organelles. We hypothesize that MaxiK surface expression is influenced by hormonal changes during pregnancy and that a retention mechanism at the sarcoplasmic reticulum (SR) or the Golgi apparatus is part of the remodeling that occurs before parturition. Here, the subcellular localization of MaxiK in isolated myometrium cells from NP, LP and post-partum (PP) mice is analyzed by confocal microscopy. The SR and the Golgi were labeled with anti-calreticulin and anti-58K-protein, respectively. Calreticulin labeling shows strong perinuclear staining typical for SR membranes, while 58K-protein staining diffusely labels more peripheral cytoplasmic regions. In LP mice myometrium MaxiK was barely detected at the plasma membrane and was highly concentrated in perinuclear regions significantly overlapping with the SR labeling. On the other hand, MaxiK labeling was distant from the Golgi labeling. Interestingly, 24 hours after parturition MaxiK labeling was absent from the perinuclear regions and was detected in clusters at the surface membrane as in NP mice. Our results indicate that MaxiK channels are primarily localized to the plasma membrane in NP mice and that towards the final stages of pregnancy MaxiK is significantly diminished from the plasma membrane and sequestered to the SR. However, the localization of MaxiK channels to the plasma membrane is rapidly restored after pregnancy suggesting that the altered trafficking signals might be induced by the expression of sex hormones during pregnancy. We propose that MaxiK is transiently stored in the SR and ready to rapidly be transported to the surface membrane after parturition.

O1-165P
HIV-1 protease inhibitory activity of *Curcuma longa*, an Indian medicinal plant.

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In 1981, an increased occurrence of unusual cases of *Pneumocystis carinii* pneumonia and Kaposi's cancer, together with other opportunistic infections was observed among previously healthy homosexual men and intravenous drug abusers. This syndrome became known as Acquired Immunodeficiency Syndrome (AIDS). Eventually in 1986, the AIDS–causing virus was given an alternative name, Human Immunodeficieny virus (HIV). During the past decade, potent agents against viral infections have become available, but the increasing clinical use has been associated with the emergence of drug resistant strains and dose-limiting toxic effects. Finding healing powers in plants is an ancient idea. During the past decade there has been increasing global interest regarding the use of medicinal plants to treat diseases. Herbal remedy for though, have been passed on from one generation to another by word of mouth, it has been scarcely studied in the light of modern medicine. The aim of the present work was to study the *in vitro* anti- Human Immunodeficiency virus activity of the plant, *Curcuma longa* extract by high performance liquid chromatography (HPLC) based HIV-1 protease inhibition assay.
Method: The plant extracts were incubated with a reaction mix containing HIV-1 recombinant protease and HIV-1 substrate (His-Lys-Ala-Arg-Val-Leu-(P-NO2-Phe)-Glu-Ala-Nle-Ser-Ser-NH2. The hydrolysate was analysed by injecting a 10 ml aliquot into the HPLC column and eluted with a gradient of acetonitrile in 0.1% TFA at a flow rate of 1.5ml/min and read at 210 nm.
Result: The roots of the plant *Curcuma longa* exhibited an inhibition of 69% against the HIV-1 protease enzyme. With the evolution of drug resistance of HIV to most of the anti-retroviral drugs, there is a need to look into other avenues. This plant extract may prove to be an alternative to the treatment of HIV. There is a need for further pharmacological and toxicological studies on this common Indian spice that is used as an Antimicrobial agent worldwide.

O1-166P
Effects of excessive iodide supplementation on intrathyroidal iodide metabolism

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We aimed to investigate influence of chronic high iodine doses (HID) treatment on thyroid functional activity. All experiments were performed on female rats Wistar, weighting 180-200 g (n=10). Rats were treated with potassium iodide (KI) 0.07, 0.21, 0.7, 7 and 35 mg/kg weight (1, 3, 10, 100 and 500 physiological doses, respectively) and after 14 days were sacrificed. As a control we used animals receiving daily physiological iodine dose. Thyroid cell functional activity was determined by measuring general, free and protein-binding tissue iodide levels, thyroperoxidase activity and serum thyroid hormone levels.
Our data indicate that chronic HID treatment leads to escape from the acute Wolff-Chaikoff effect. Firstly, 1.60, 1.86, 1.86, 2.21 and 1.76-fold general iodide levels (KI 0.07, 0.21, 0.7, 7 and 35 mg/kg, respectively) and 1.95, 2.28, 2.13 and 2.02-fold free iodide levels (KI 0.21, 0.7, 7 and 35 mg/kg) rise evidences iodide uptake activation. Secondly, this accompanied by iodine oxidation activation: HID 1.57, 1.35, 1.43, 2.08 and 1.42-fold increased the protein-binding iodide levels (KI 0.07, 0.21, 0.7, 7 and 35 mg/kg, respectively). Thirdly, we also found secretory function normalization of rat thyroid. Since serum T4 and T3 levels showed no differences from that in control animals. There is no significant altering of thyroperoxidase activity under chronic HID supplementation conditions with exception of 3.42-fold decreasing in KI 35 mg/kg treated groups.
Our results suggest that thyroid adapted to chronic HID treatment. Escape from the acute Wolff-Chaikoff effect leads to normalization thyroid hormones levels that probably associated with protein-binding iodide levels increasing.

O1-167P
Expression of CD44v3 in human melanoma is associated with metastatic propensity in experimental models and clinical samples

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CD44 is a family of adhesion molecules of several different splice variants, the function of which is further modulated by glycanation of the extracellular domain. Although the majority of the CD44 splice variants is chondroitin sulphate proteoglycan, the v3 variant is a part-time heparan sulphate proteoglycan with unique functional properties ranging from matrix adhesion to cytokine-binding. We have analyzed the CD44 splicing patterns of human melanoma cell lines *in vitro* and in metastatic SCID mice models by RT-PCR. We have identified a "melanoma-specific" CD44v expression pattern from 10 *in vitro* growing human melanoma cell lines by PCR technique using 5 different primer pairs designed to the variable region of CD44. This *in vitro* pattern was independent from the matrix milieu of the cells and was different from those of the epidermoid- and colon carcinoma or Kaposi sarcoma. In the following, we have examined the changes of this expression pattern in the metastatic and non-metastatic primary tumors of three genetically different cell lines growing in scid mice. Our results indicated that the overexpression of CD44v3 variant correlated with the metastatic phenotype on the basis of q-PCR experiments. Analysis of 46 primary human melanoma samples indicated that a significant proportion expressed the CD44v3 protein (15/46). Five year follow-up of the patients revealed that 21 cases progressed to visceral disease and the majority of them expressed CD44v3 (14/21), accordingly, these patients had significantly shortened survival. Finally, we have made an attempt to identify the function of the CD44v3 molecule in the melanoma metastasis. We have found that a monoclonal antibody against CD44v3 inhibits *in vitro* migration of human melanoma cells suggesting the involvement of this splice variant in the invasion process. (This work was supported by the Ministry of Education, NKFP 1/48/2001.)

O1-171P
Elevated Src kinase activity results in increased protein tyrosine phosphorylation in scrapie-infected neuronal cell lines

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Prion diseases are fatal neurodegenerative diseases that occur in a wide variety of mammals, including Creutzfeldt-Jakob disease in humans and scrapie in sheep. Prion diseases are characterized by accumulation of the abnormal prion protein (PrPSc), astrogliosis and neuronal spongiform degeneration, yet the biochemical and cellular changes in prion diseases remains largely unknown. The host-encoded prion protein PrPC is a glycosyl-phosphatidylinositol (GPI)-anchored protein, localized to sphingolipid- and cholesterol-rich membrane domains or 'lipid rafts'. Lipid rafts are highly specialized microdomains, believed to function as platforms for the attachment of lipid-modified proteins e.g., GPI-anchored proteins and src-family tyrosine kinase, as well as to group, modulate and integrate signaling events at the cell surface.
Because both PrPC and PrPSc are localized to rafts we have studied how the formation and accumulation of PrPSc in rafts may affect the Src kinase activity in two different scrapie-infected neuronal cell lines: ScGT1 and ScN2a, compared to their noninfected counter parts. By immunoblotting, using clone 28 – a monoclonal antibody specific for the active form of Src and Fyn kinases, we have found increased levels of both active Src and Fyn in ScGT1 and ScN2a cells. *In vitro* kinase assay confirmed the increased Src activity. Comparing the amount of active Src and Fyn to the amount of Src and Fyn protein levels, revealed an increased specific activity of Fyn, whereas the increased Src kinase activity was correlated to an elevated Src protein level. In addition an important increase in protein tyrosine phosphorylation was observed in ScGT1 and ScN2a cells, which was further shown to be Src-dependent, as treatment with PP2- a Src family kinase specific inhibitor, completely reversed the protein tyrosine phosphorylation profile.
Although several studies have shown that PrPC may interact with and/or activate Src family kinases, our study shows for the first time that abberantly expressed and regulated Src kinase activity result in increased protein Tyr phosphorylation in scrapie-infected neuronal cells. Abnormal Src-kinase activation and subsequent protein tyrosine phosphorylation may be key elements in the neuropathology of the prion diseases.

O1-172P
The origin of membranes that emancipated mineral imprisoned cells are components of ganti´s chemoton in a chemically oscillating system

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The geochemical machine that we are proposing for the origin of life has at its heart Ganti´s chemoton [Ganti, 1966, 1971, 2003] and owes much to Wächtershäuser´s [1988] surface metabolism that derive energy from the oxidation-reduction reaction of $FeS\text{-}H_2S/FeS_2$. In our theory the first spur of energy comes from surface metabolism attached to the pyrite. After this first moment the chemoton takes over and creates

cycles that base their movement to stoichiometric chemistry. Thus, when contemplating the origin of life, we deal with the appearance of at least three different but in the remote past often convergent natural systems: (1) the operation of a non evolutionary self organizing synergic system [Haken, 1983], (2) within it the chemically fluid machine which in Ganti´s term is the result of a chemically oscillating system, and (3) as the result of such fluid machines cycles are produced that later manifest Lamarkian and Darwinian processes with the most sophisticated chemoton. For this theory we accept the existance of various transitions in the evolutionary process [Maynard-Smith and Szathmáry, 1995]. Our theoretical exploration place us in the first level of natural selection where cellular entities are very primitive, deprived of real individuality; they are simple primary units of selection in which replicators compete in the most Darwinian manner, without any cooperation or interactions among genes [Hoenigsberg, 2003]. In this first level of selection the hereditary mechanism is still incomplete and although with a template mechanism for copying information (in this primitive world there is no RNA because we propose the presence of lipozymes capable of catalysis and of guarding information [as in Fendler and Fendler, 1975]. The manifestation of life in the first instance was a lame biological-chemical hybrid with expressions of both systems bound as an uncertain entity. This new hybrid slowly casts-off its chemical 'skin' to jump to a new level of selection in the road that were to take it to the dictum... Natural selection requires heritable variations in fitness, and genes as a programme-controlling compartment serving as a centralized unit of information.

O1-174P
Oxidative modification of rat thyroperoxidase

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Thyroperoxidase (TPO) is a membrane-bound heme-containing glycoprotein that catalyzes the synthesis of thyroid hormones in the presence of H_2O_2. In the present study, we examined the effects of oxidative stress activation on the TPO activity. The experiments were carried out on a rat thyroid microsomal fraction (MF). TPO activity was measured by iodide assay. It was shown that the incubation of thyroid MF with a prooxidants (Fe^{2+}/ascorbate) decreased TPO activity (by 75.0% after 30 min) that accompanied by 3-fold increasing of lipid peroxidation levels in the reaction medium. The inhibition of TPO was shown after the incubation of thyroid MF with H_2O_2 and malondialdehyde. The H_2O_2 concentration 10 nmol/l – 10 mmol/l led to TPO inhibition by 10.0-64.5%; 0.1 mol/l H_2O_2 completely inactivated TPO. Malondialdehyde at the concentration of 0.05-0.5 mmol/l decreased TPO activity by 24.3-82.7%. The incubation with Fe^{2+}/ascorbate and malondialdehyde led to change in the time course of the enzyme reaction that was characterized by a distinct lag-period. The lag-period of the enzyme reaction increased depending on the time of incubation with Fe^{2+}/ascorbate and the concentration of malondialdehyde. It is known, that a radiation effect is primarily manifested by activation of free radical production. We compared the enzyme kinetics of TPO from thyroid MF from control and irradiated (1 Gy) rats. There was no change in apparent K_m for H_2O_2. The apparent K_m value for KI was 71.8 microM for TPO from thyroid MF of the irradiated rats, with 1.8-fold exceeds the control Km for KI (38.6 microM), indicating decreased substrate affinity. We conclude that reactive oxygen species and lipid peroxidation products induced modification and inhibition of thyroperoxidase.

O1-175P
The evaluation of performances of two different homocysteine measurement methods used in clinical laboratories

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It has been proposed that the homocysteine produced during the metabolism of methionine plays a role in etiopathogenesis of several diseases. The interest in measurement of plasma homocysteine levels has increased recently upon the finding that homocysteine is an independent risk factor for cerebrovascular and coronary artery diseases.
In the present study, we measured plasma homocysteine levels with two different systems; Chromsystems kits in HP1100 and Drew DS30 and investigated the analytical performances of these two systems with respect to homocysteine measurements in clinical laboratories. Reproducibilities for Drew DS30 and Chromsystems kits in HP1100 were 2.1% and 1.6%, respectively (p=0.043). Inter-assay precisions for low (5 µmol/L), medium (12.5 µmol/L) and high (24.8 µmol/L) homocysteine levels were 4.9 vs. 4.0% (low), 5.2 vs. 4.2% (medium), and 5.8 vs. 4.2% (high) for Drew DS30 and Chromsystems kits, respectively. Homocysteine levels measured with these two systems showed a good correlation (r = 0.948). Linearity limits for Drew DS30 and Chromsystems kits were 100 µmol/L and 200 µmol/L, respectively.
The findings of the present study suggested that the use of Chromsystems HPLC for homocysteine measurement might be more useful both in groups with predicted hyperhomocysteinemia and in laboratories with high number of patients where there are limitations in sample amounts, and working hours. On the other hand, in laboratories without these limitations, DS30 system might be used with a similar performance to Chromsystems kits.

O1-176P
SAGA-associated histone deubiquitinase represses H3-K4 methylation differentially *in vivo*

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Despite recent advances in characterizing the repression of H3 (histone 3)-K4 (lysine 4) methylation at the GAL1 gene by H2B-K123 specific deubiquitinase activity of yeast SAGA (Spt-Ada-Gcn5-acetyltransferase)-associated Ubp8p, our knowledge of the general role of Ubp8p at the SAGA-dependent genes is lacking. Here, using a formaldehyde-based *in vivo* cross-linking and chromatin immunoprecipitation assay, we have systematically analyzed the role of Ubp8p in regulation of H3-K4 methylatyion at several SAGA-dependent yeast genes, namely, PHO84, ADH1, CUP1 and INO1. Like GAL1, we show that H3-K4 methylation is increased at the PHO84 core promoter in the UBP8 deletion mutant. Interestingly, H3-K4 methylation remains invariant at the PHO84 ORF (open reading frame) in Δubp8, demonstrating a highly localized role of Upb8p in repression of H3-K4 methylation *in vivo*. However, unlike PHO84, H3-K4 methylation at three other SAGA dependent genes is not controlled by Ubp8p. Furthermore, we show that Ubp8p and H3-K4 methylation are dispensable for PIC (pre-initiation complex) assembly at the core promoters of these genes. Collectively, Ubp8p differentially controls H3-K4 methylation at the SAGA-dependent promoters, revealing a complex regulatory network of histone methylation *in vivo*.

O1-179P
An *ab initio* quantum chemical investigation of solventinduced effect on 14N-nqr parameters of alanine, glycine, valine, and serine using polarizable continuum model

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In this paper *ab initio* quantum mechanical calculations of the 14N-NQR parameters of alanine, glycine, valine and serine obtained from electric field gradient (EFG) tensor such as quadrupole frequencies and asymmetry parameters in gas phase and different solvents have been carried out with Gaussian 98 software package and solvent-induced effect on these parameters have been evaluated using density functional theory (DFT). Furthermore, direct and indirect solvent effects on asymmetry parameters have been also calculated. We found out that NQR parameters of nitrogen atoms of amino acids are highly sensitive to environmental effects and observed solvent-induced shielding variation is strongly related to the values of dielectric constants of the solvent and whether it is protic or aprotic. For more investigation of the solvent effect, the relative energies of each amino acid in various solvents have been calculated and the graphs of the relative energies verses dielectric constants have been analyzed.

O1-181P
Systematic discovery of short linear motifs

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Many aspects of cell signalling, trafficking and targeting are governed by interactions between globular protein domains and short peptide segments. These domains often bind multiple peptides that share a common sequence pattern, or linear motif (e.g. SH3 binding to PxxP). Many domains are known, though comparatively few linear motifs have been discovered. Their short length (3-8 residues), and the fact that they often reside in disordered regions in proteins makes them difficult to detect through sequence comparison or experiment. Nevertheless, each new motif provides critical molecular details of how interaction networks are constructed, and can explain how one protein is able bind to very different partners. Here we show that real motifs can be detected using data from genome-scale interaction studies, and thus avoid the normally slow discovery process. Our approach, based simply on motif over-representation in non-homologous sequences, re-discovers known motifs and predicts dozens of others. Direct binding assay reveals that two predicted motifs (of three tested) are indeed protein-binding modules: a DxxDxxxD Protein Phosphatase 1 binding motif with a KD of 22 microM and a VxxxRxYS motif that binds Translin with a KD of 43microM. We estimate that there are dozens or even hundreds of linear motifs yet to be discovered that will greatly illuminate cellular processes.

O1-184P
Analysis of binding site residues and ligands in membrane protein-ligand complexes

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Membrane proteins are involved in key roles in various biologically important functions such as biological transduction and transport processes by mediating the flows of ions, energy and information across membranes. The structural analysis on the interactions between the membrane proteins and ligands provides deep insights into the mechanism of protein-ligand interactions and their functions. An increasing number of experimentally determined structures of proteins would help to derive the structural principles in protein-ligand complexes. In this work we have analyzed the membrane protein-ligand interactions from the information available in PDB and other structural databases. The interactions between amino acids residue in membrane protein and the functional group of the ligand have been analysed through atom contact, hydrophobic behavior, location of the residue, etc. The preliminary atom contact analysis on 294 membrane protein-ligand complexes comprises of 1174 active/binding sites shows that 7.23%, 27.91% and 64.86% of atom contacts are around the ligands at the vicinity of 0-3.0, 3.0-4.5 and 4.5-6.0 Å which reveals the fact that, in addition to the direct interactions, the influence of the neighboring atoms may also play key roles in the binding of these ligands at the binding site. Additional calculations and development of database and more interesting results thus obtained upon the analysis will be presented.

O1-185P
Comparative analysis of two independent mutants in AtBRM gene in *Arabidopsis thaliana*.

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In multicellular organisms, remodeling of chromatin structure plays a key role in controlling gene expression and in maintaining specific transcriptional states during development. We present results of comparative analysis of insertional mutant in the AtBRM gene encoding a homolog of the SWI2/SNF2 ATPase: a catalytic subunit of the SWI/SNF-type chromatin remodeling complex. In this mutant the insertion abolishes the transcription of the gene resulting in a null mutation. Characterization of the effects of this mutation suggests that the *Arabidopsis* homolog of a key chromatin remodeling ATPase plays vital role in determining an overall plant architecture, leaf and flower development, the transition from vegetative to reproductive phase of growth and plant fertility.

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Glycoproteomics: Protein Modifications for Versatile Functions, June 28-30, 2005, Dubrovnik, Croatia (Gordan Lauc, glauc@pharma.hr)

Biological Electron Transport in Respiration and Photosynthesis: Structure and Function of Bioenergetically Relevant Proteins, June 28-July 2, 2005, Schloßhotel Wilhelminenberg, Vienna, Austria (Günter A. Peschek, guenter.peschek@univie.ac.at)

Protein Folding and Transport in Health and in Disease, June 28-July 2, 2005, Bucharest, Romania (Stefana Petrescu, email: stefana@biochim.ro)

9th International Symposium on Proteinase Inhibitors and Biological Control, June 26-June 29, 2005, Brdo Estate (near Ljubljana Airport), Slovenia (Boris Turk and Marko Dolinar, email: boris.turk@ijs.si)

PLANNED SPECIAL EVENTS:

- Youth Program
- Meet the Entrant — Meet the Expert
- Youth Excellence Awards
- Science Education
- Teaching of Biochemistry
- Media Relations
- Public Awareness Program
- Women in Science
- Intellectual Property Rights
- When your Proteins get Smuggled: a Marvel Lecture Session
- The Best Protein Cook Contest "The Most Beautiful Protein"
- Sculpture and 3-D Modeling Contest
- The Sound of Proteins Contest
- Lift your Proteins to the Air. FEBS IUBMB Balloon
- Hungarian Protein Products Fair

SECRETARIAT:
2005 FEBS Congress and IUBMB Conference
Chemol Travel Congress Department
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STREET ADDRESS: H-1052 Budapest, Deák Ferenc str. 10., Hungary
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