

D1 – Extracellular Matrix Proteins

D1-001

Sox9 controls both chondrocyte differentiation and proliferation

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The transcription factor Sox9, which is required to establish the chondrocyte lineage, has essential functions at several steps throughout the chondrocyte differentiation pathway. Sox9 is needed at an early step that of mesenchymal condensation. It is then needed for the overt differentiation of chondrocytes in part because Sox9 is required during chondrogenesis for expression of Sox5 and Sox6, which in turn have an essential role at this step. Sox9 also inhibits the maturation of chondrocytes into hypertrophic chondrocytes. Finally, our experiments indicate that Sox9 is needed to establish osteochondroprogenitors. We hypothesize that at each step the mechanisms by which Sox9 exerts its function are different. Furthermore, work by other laboratories as well as ours have indicated that Sox9 also has an essential role in cell fate decisions in several other lineages, including glial cells in the central nervous system, cells in the male gonads, cells in the endocardial cushions, which are the precursors of the heart valves and septa, cranial neural crest cells. We speculate that other factors are needed for the specificity of Sox9 at each step of chondrocyte differentiation as well as for its role in other cell lineages. We have also shown that Sox9 physically and functionally interacts with β -catenin and inhibits the transcriptional activity of β -catenin. These interactions target β -catenin for proteasome degradation. Furthermore, either overexpression of Sox9 or inactivation of β -catenin in chondrocytes causes a similar phenotype of dwarfism with decreased chondrocyte proliferation, and delay in hypertrophic differentiation and endochondral bone formation. In addition, either inactivation of Sox9 or abnormal stabilization of β -catenin in chondrocytes also produces a similar phenotype of severe chondrodysplasia. These results suggest that chondrogenesis is controlled by interactions between Sox9 and the Wnt/ β -catenin signaling pathway. We propose that, in addition to its essential role in several steps of chondrocyte differentiation, the ability of Sox9 to control cell proliferation represents a crucial property of this differentiation factor.

D1-002

The role of chondrocyte-matrix attachment complexes in skeletal development

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Skeletogenesis and bone homeostasis crucially depend on cell adhesion to the extracellular matrix (ECM) for differentiation, migration, proliferation and survival of bone and cartilage cells. In general, cell adhesion to ECM molecules such as collagens and fibronectin is largely mediated via the integrin family of transmembrane receptors. Integrin function is modulated via the recruitment of cytoplasmic plaque proteins, which form molecular complexes (platforms) at the cell-matrix attachment sites and initiate signal transduction pathways. One such a molecular platform interacting with b1 integrin includes the association of the

Integrin-linked Kinase (ILK)-PINCH-1 (particularly interesting new cysteine histidine-rich protein)-parvin complex. To address the role of this platform during endochondral bone formation (EBF), we have generated mouse strains lacking some players of the complex in chondrocytes. The b1 integrin mutant mice develop a severe chondrodysplasia characterized by abnormal cell shape and impaired chondrocyte motility, survival, proliferation and cytokinesis. Mice lacking fibronectin, the substrate of a5b1 integrin, in cartilage display normal skeleton suggesting a pivotal role of collagen-binding integrins in EBF. To prove this hypothesis, we ablated the a10 integrin gene diminishing the major collagen-binding integrin a10b1 on chondrocytes. a10-null mice develop a mild chondrodysplasia characterized by a moderate disorganization of the growth plate and reduced proliferation of chondrocytes. Finally, chondrocyte-specific deletion of ILK leads to reduced proliferation and changes in cell shape suggesting that ILK mediates some but not all b1 integrin function in chondrocyte. Altogether these findings establish that integrin-mediated chondrocyte-ECM interactions are required for multiple steps of EBF.

D1-003

The matrilins – adaptor proteins in the extracellular matrix

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The matrilins form a four-member family of modular, multisubunit matrix proteins, which are expressed in cartilage but also in many other forms of extracellular matrix. They participate in the formation of fibrillar or filamentous structures in the extracellular matrix and are often associated with collagens. It appears that they mediate interactions between collagen-containing fibrils and other matrix constituents, such as aggrecan. This adaptor function may be modulated by physiological proteolysis that causes the loss of single subunits and thereby a decrease in binding avidity. Attempts to study matrilin function by gene inactivation in mouse have been frustrating and so far not yielded pronounced phenotypes, presumably because of the extensive redundancy within the family allowing compensation by one family member for another. However, mutations in matrilin-3 in humans cause different forms of chondrodysplasias and perhaps also hand osteoarthritis. As loss of matrilin-3 is not critical in mouse, these phenotypes are likely to be caused by dominant-negative effects.

D1-004

The central role of integrin-mediated adhesion in controlling epithelial cell survival and differentiation

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Much of our current work focuses on understanding how breast epithelium develops and functions, and how alterations in normal

cellular homeostasis might provide a molecular basis for breast cancer. We are interested specifically in the mechanisms by which epithelial cell adhesion to external structural molecules, i.e. the extracellular matrix, regulates their behaviour. Integrins are a class of adhesive transmembrane receptors that organize cellular architecture and control cell migration and intracellular signalling processes, via adhesion-activated enzymes such as focal adhesion kinase and integrin-linked kinase. These and other components within the adhesion-signalling system regulate mammary gland development *in vivo*, as well as the survival and differentiation of breast epithelial cells in culture models. We have identified signalling pathways through which integrins control both hormonal-dependent epithelial differentiation, as well as the programme of mitochondrially driven apoptosis. In this talk I will explore two facets of our work. First, I will discuss studies to investigate molecular basis of integrin-hormone receptor cross talk using both adenoviral-mediated gene transfer studies with dominant-negative integrin-signalling proteins, and mammary cell cultures from mice harbouring floxed alleles of integrin-signalling genes. Secondly, we have identified a novel function for integrins, which is to control shuttling of the pro-apoptotic protein, Bax, between the cytosol and mitochondria, and I will discuss data indicating that focal adhesion kinase, protein kinase B and p21-activated protein kinase-1 are essential components in this pathway.

D1-005

The extracellular matrix 1 gene is essential for early mouse development

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Introduction: The human extracellular matrix 1 gene (ECM1) encodes a 85 kDa glycoprotein with a cysteine distribution [CC(X7-10)C] comparable with that of the serum albumin proteins. The mechanism by which the ECM1 protein exert its biological function is still unknown. Based upon the ECM1 expression pattern and the effects of recombinant ECM1 protein on different *in vitro* model systems ECM1 is likely to play a role in endochondral bone formation, epidermal differentiation and angiogenesis. ECM1 interacts with perlecan that plays an important role in skin and bone development [1].

Method: To investigate the *in vivo* role of ECM1, we used homologous recombination in mouse embryonic stem cells to produce ECM1 null mice by deleting the first two exons of the mouse ECM1 gene (thus deleting the transcription and translation start). Two independent ECM1 KO mice lines were generated.

Results: Mice homozygous for the ECM1 null mutations (ECM1^{-/-}) are not viable and mutant embryos die around implantation, before the onset of gastrulation. Heterozygous mice (ECM1^{+/-}) are fertile and indistinguishable from wild-type littermates. Expression studies (RT-PCR) revealed that embryonic ECM1 is already expressed from mouse pre-implantation development (E4.5) onwards.

Conclusions: The ECM1 null phenotype demonstrates an unexpected and crucial role for ECM1 during early stages of mouse development. Experiments are being performed to elucidate the role of ECM1 during pre-gastrulation development. The early embryonic lethality prevents however, to study in this mouse model the function of ECM1 in, e.g. skin or bone development.

Reference

- Chan. The role of extracellular matrix protein 1 in human skin. *Clin Exp Dermatol* 2004; **29**(1): 52–56 (Grant identification: FWO: G.0133.05).

D1-006

A novel COCH mutation, V104del, impairs folding of the LCCL domain of cochlin and causes progressive hearing loss

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Autosomal dominant non-syndromic sensorineural deafness (DFNA9) is a rare, late onset progressive hearing loss. In DFNA9 patients, hearing loss usually begins in the third decade of life affecting higher frequency ranges with concomitant vestibular dysfunction. The patients show accumulation of eosinophilic deposits in vestibular and cochlear nerve channels. DFNA9 is caused by mutations in the COCH gene, which encodes cochlin, an extracellular matrix protein that contains an LCCL domain and two von Willebrand type A domains. The biological function of the protein is unknown. Molecular analysis of DFNA9 cases has identified six different mutations in this gene. All mutations causing DFNA9-type deafness affect the LCCL domain of cochlin. Our investigation was aimed at identifying novel mutations affecting the LCCL domain of cochlin in order to gain more insight into the pathomechanism of DFNA9. This study describes a novel COCH mutation in a Hungarian patient, which results in the deletion of Val104, a residue conserved in the human, mouse and chicken cochlin sequences. The deletion affects a critical β strand of the LCCL domain and was found to prevent refolding of a recombinant LCCL domain expressed in *Escherichia coli*. In this respect, the V104del mutation is similar to most other DFNA9 causing mutations identified so far, which also impair refolding of the recombinant LCCL domain. This novel mutation provides additional support for the former notion that the characteristic deposits in the inner ear structures could be the result of accumulation and aggregation of aberrant, mutated cochlins over a longer time course. It is consistent with the late onset and progressive nature of this disorder.

D1-007P

Establishment of double transgenic mice for investigating the relationship of decorin and TGF- β 1

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Fibrosis, the accumulation of connective tissue in various chronic diseases, severely impairs the affected organ. Thus, inhibition of fibrogenesis is a chief therapeutic aim in the treatment of these disorders. TGF- β , one of the major stimulators of fibrogenesis, is the principal target of antifibrotic research. Decorin is also known to regulate the proliferation of the connective

tissue. Its protective effect against renal fibrosis has already been confirmed. To investigate the role of decorin in liver fibrogenesis and cancer, we created an *in vivo* mouse model. Decorin^{-/-} animals (Dec^{-/-}) were mated with TGF-β1 transgenic mice overexpressing the growth factor in their liver (TGF-β1OE). The double transgenic mice (Dec^{-/-}/TGF-β1OE) have no decorin gene, and are hemizygotic for active TGF-β1, carrying the gene on chromosome Y. The liver of both the Dec^{-/-} and Dec^{-/-}/TGF-β1OE animals seemed healthy in gross appearance, while TGF-β1OE mice were reported to develop spontaneous fibrosis at early age. Immunohistochemically, a decreased level of collagen type IV was found in liver of the Dec^{-/-} animals. The most salient feature of Dec^{-/-} and Dec^{-/-}/TGF-β1OE mice is the appearance of hairless areas on their skin. As we expected an altered response of transgenic mice to chronic liver injury, cirrhosis and cancer have been induced by thioacetamide and diethylnitrosamine. According to our preliminary results, thioacetamide provoked severe cirrhosis in both Dec^{-/-} and Dec^{-/-}/TGF-β1OE animals. In addition, multifocal tumours developed in the Dec^{-/-} mice but not in the Dec^{-/-}/TGF-β1OE animals. In the future, we try to elucidate the mechanisms underlying these phenomena.

D1-008P

Effect of high hydrostatic pressure on osteoinductive properties of extracellular bone matrix proteins

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In orthopedic surgery, sterilization of bone used for reconstruction of osteoarticular defects caused by malignant tumors is carried out in different ways. At present, to devitalize tumor-bearing osteochondral segments, mainly extracorporeal irradiation or autoclaving is used. Both methods have substantial disadvantages, e.g. loss of biomechanical and biologic integrity of the bone. In particular integration at the autograft–host junction after reimplantation is often impaired due to alterations of the osteoinductivity following irradiation or autoclaving. As an alternative approach, high hydrostatic pressure (HHP) treatment of bone is a new technology, now being used in pre-clinical testing to inactivate tumor cells without alteration of biomechanical properties of bone, cartilage and tendons. The aim of this study was to investigate the influence of HHP on fibronectin (FN), vitronectin (VN), and type I collagen (col. I) as major extracellular matrix proteins of bone tissue, accountable among others for the osteoinductive properties of bone. Fibronectin, vitronectin, and type I collagen were subjected to HHP (300 and 600 MPa) prior to the coating of cell culture plates with these pre-treated proteins. Following the biologic properties were measured by means of cell proliferation, adherence, and spreading of the human osteosarcoma cell line (Saos-2) and primary human osteoblast-like cells. Up to 600 MPa all tested matrix proteins did not show any changes, regarding the biologic properties adherence, spreading and proliferation. We anticipate that, in orthopedic surgery, HHP can serve as a novel, promising methodical approach, by damaging normal and tumor cells

without alteration of osteoinductive properties, thus facilitating osteointegration of the devitalized bone segment in cancer patients after reimplantation.

D1-009P

Purification of decorin core protein from human lung tissue

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Aim: Extraction and purification of decorin core protein from human lung tissue.

Background: Decorin is an important member of the leucine-rich repeats proteoglycans found also in lung extracellular matrix (ECM). Diminished immunohistochemical staining of decorin in patients with smoking-related emphysema has been reported. This fact may indicate that degradation and/or structural modification of decorin is related to disease development. In order to investigate the role of decorin in emphysema development and to establish if the immunohistochemical changes seen in emphysema patients are due to a decreased expression or a qualitative alteration (e.g. degradation) of the core protein, we isolated decorin from human lung tissue.

Method: Anion-exchange chromatography (AEC) was used to enrich the negatively charged proteoglycans. After deglycanation by treatment with chondroitinase ABC, proteoglycan core proteins were recovered in the flow through when run into the same AEC. Decorin core protein was isolated by reverse-phase HPLC on a C4 column.

Results: Our method allows the purification of decorin core protein from lung tissue. After last step of chromatography, the corresponding decorin peak gives a single band when analyzed by SDS-PAGE. Decorin enrichment was monitored by Western blot and SDS-PAGE and its identity was confirmed by in-gel digestion and mass spectrometry.

Future prospects: Fine structural comparison of the protein core (in terms of glycosaminoglycan-protein linkage region and the three N-linked oligosaccharides attached near the C-terminus) between normal and pathologic state will be performed, in order to reveal possible alterations related to disease progression.

D1-010P

The function of the unique module of matrilin-2: alternative splicing and proteolytic processing result in variation of the oligomeric structure

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Matrilin-2 is a component of collagen-associated and -independent extracellular filamentous networks. By itself it forms oligomers of variable sizes. Two isoforms of matrilin-2, differing from each other by the presence or absence of 19 amino acids in the

center of the unique module, are translated from alternatively spliced mRNAs. To study the molecular basis of oligomer formation truncated forms of matrilin-2 were expressed in human embryonic kidney 293 cells and purified from conditioned medium. The recombinant proteins showed varying degree of oligomerization as a consequence of coiled-coil assembly, disulfide bridge formation and proteolytic processing. All three constructs contained the second wWFA domain and the coiled-coil and preferred to form trimers. Presence of the longer version of the unique module resulted in adjoining of the trimers to form multimers. The shorter version of the unique module did not facilitate multimerization, however proteolytically processed matrilin-2 species were also detected. The cleavage occurs in the unique region, most probably at multiple sites leading to the release of large fragments and the formation of dimers and monomers of intact subunits still containing a trimeric coiled-coil. In immunoblots of tissue extracts similar degradation products could be detected, indicating that a related proteolytic processing occurs *in vivo*. The truncated version without the unique segment formed a trimer and showed minimal proteolytic cleavage. The diversity of the matrilin-2 oligomeric forms may be important for the formation of filamentous network by enhancing structural and functional complexity.

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D1-011P

Structural analysis of glycosaminoglycans and proteoglycans from fresh porcine aortic valves

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Cardiac valves are specialized forms of cardiovascular connective tissue that are designed to support high bearing and shearing stresses during their function. Mechanical properties of extracellular matrix are critically important for performance and durability of heart tissue valve substitutes. It has been reported that extracellular matrix damage during valve substitute processing may result in increased valve structural failure and calcification. The valve consists of a semifluid, deformable, avascular matrix rich in proteoglycans (PGs), glycosaminoglycans (GAGs) and collagen fibres. Our previous studies indicated that localized GAG depletions could be produced following cryopreservation protocols. Moreover, GAG distribution suggested some regional specializations. The present investigation was undertaken to study GAG and PG content, distribution and structure in three different zones (aortic wall, valve commissure and leaflet) of fresh porcine aortic valve. The three selected areas were significantly different in GAG total content. GAG analysis indicated that deep structural dissimilarities in polysaccharide chains exist, depending on their topographic localization. In particular, commissure and leaflet, compared with aortic wall, showed a higher relative percentage of hyaluronan and the presence of a peculiar undersulphated chondroitinsulphate (slow CS). Structural analysis suggested that these differences concern both the iduronation and the sulphation degree of CS. Our findings regarding fresh porcine valve GAG composition, together with evidence of topographic changes in the relative amount of specific constituents, provide a basis on which to analyse the integrity of extracellular matrix in both tissue valve substitutes and decellularized scaffolds.

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D1-012P

Growth factor-dependent inhibitory effect of genistein on the biosynthesis of chondroitin / heparan sulfate proteoglycans and hyaluronan in fibrosarcoma cells

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The inhibitory effect of genistein in cancer cell growth, proliferation, and metastasis has been reported in breast cancer, in prostate cancer, osteosarcoma, and fibrosarcoma. Glycosaminoglycans (GAGs)/proteoglycans (PGs) components of ECM have an important functional role in the cell proliferation and/or differentiation. The aim of this study was to examine the effect of genistein on the basal and growth factor-induced GAG biosynthesis in fibrosarcoma cells. We utilized the fibrosarcoma cell line B6FS and assayed the effects of bFGF, TGF α and PDGF-BB combined with the genistein treatment. The identification of cell bound and secreted GAGs/PGs was accomplished after metabolic labeling by a modification of the HPLC technique. It was found that the majority of GAGs in cultured medium was HA in a percentage of 69.46%. In the cell fraction HS was found to be 63.1% of total cell bound GAGs/PGs B6FS. Treatment with genistein at 10 μ g/ml inhibited ca. 80% both cell associated and secreted GAGs/PGs, and treatment with 30 μ g/ml genistein resulted in almost complete (>90%) inhibition of biosynthesis of all GAGs/PGs. The inhibitory effect of genistein demonstrated no GAG-subclass specificity. Combined treatment with genistein and bFGF or PDGF resulted in a net inhibitory effect on secreted and cell bound GAG biosynthesis by a percentage of 80% (10 μ g/ml genistein) suggesting that these growth factors cannot overcome the genistein inhibitory activity. Nevertheless, the combined treatment with genistein and TGF α resulted in a partial recovery of the GAG biosynthesis suggesting that TGF α employs both PTK-dependent and -independent cell-signaling pathways. In conclusion, the results demonstrate a highly inhibitory activity of genistein on GAG biosynthesis in fibrosarcoma cells which cannot be overcome by bFGF, PDGF and only partially by TGF α and help to delineate further the mechanism of the antitumor action of genistein which has been reported in the fibrosarcoma cancer model.

D1-013P

The role of MMPs in the progression of periodontitis in Portuguese individuals

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Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases that can virtually cleave all structural extracellular matrix (ECM) molecules, acting as key mediators in inflammation and in matrix remodelling, besides having the ability to process bioactive molecules such as growth factors and cytokines, cell surface receptors and adhesion molecules. Periodontitis is a bacterially induced chronic

inflammatory disease, in which the gingival pocket epithelium proliferates extensively and grows into the periodontal connective tissue coinciding with ECM degradation and loss of tooth attachment. The final aim of this work deals with the role of MMPs in periodontitis in a Portuguese population (from Beira Interior), using saliva and gingival crevice fluid samples. Given that the human saliva contains a large number of proteins that can be used for a series of diagnosis, this first approach aims to establish an extraction protocol for pro- and active-forms of MMPs, to be analysed by oriented proteomics, namely 2D-zymography. One of the problems to overcome is saliva viscosity that is due, among others, to the fact that the supramolecular pellicle precursors are unstable and reach a thermodynamically more favourable state by adhesion to a solid surface. Their micellar nature suggests the use of detergents as dissociation agents of the salivary globes, and therefore two detergents were tested: Triton X-100 and IGEPAL. The selected method uses the first detergent, which utilization does not separate the typical high-molecular weight aggregate usually found on zymography of saliva samples. The contents of these aggregates are being characterized, concerning the presence of MMPs, their tissue inhibitors, and α_2 -macroglobulin.

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D1-014P

Developmental expression and function of neurocan in the early chick embryo

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Neurocan, a chondroitin sulfate (CS) proteoglycan, interacts with other molecules of the extracellular matrix (ECM) and the cell surface and participates in signaling pathways. Relatively little is known about the neurocan tissue-specific distribution or function during development. The expression pattern of neurocan was examined by immunofluorescence/immunoprecipitation of chick embryo from stage X (morula) up to stage HH17 (29 somites/organogenesis). The chick embryo at stages X, XIII (blastula), and HH3-4 (primitive streak/gastrula) did not show neurocan immunoreactivity. Neurocan was first detectable in cells in the inchoate neural plate and in the ECM in embryos at stage HH5 (early neurula). In embryos at stage HH13 (19 somites), neurocan fluorescence was intense in the brain, notochord, in the foregut lower wall, in dorsal mesocardium and myocardium but there was no fluorescence in the endocardium. The dermamyotome showed strong while the sclerotome weaker neurocan fluorescence in somites. The pre-migratory neural crest cells showed intense fluorescence but the migrating neural crest cells showed no immunoreactivity. At stage HH17 (29 somites) the time when the first neurons begin to differentiate, neurocan expression was intense in the brain, the foregut lower wall, the mesonephros and in blood islands. Expression of neurocan was strong in the retina, weaker in lens and intense in the cornea in eyes and intense in the myocardium in heart. On sodium dodecyl sulfate polyacrylamide gel electrophoresis, neurocan is a 220 kDa core protein and is linked to CS side chains. Blocking antibodies to neurocan resulted in abnormal brain development and inhibited somite and heart morphogenesis.

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D1-015P

Studying the role of macrophage migration inhibitory factor in aseptic loosening of arthroplasties

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Introduction: The predominant complication concerning total joint replacement operations still remains the aseptic loosening. Many cytokines, prostaglandins and proteolytic enzymes, especially MMPs, are released in periprosthetic tissues, causing osteolysis and therefore resulting in loosening of endoprostheses. Macrophage migration inhibitory factor (MIF) is a cytokine with catalytic activities, produced by T cells, macrophages and other cells. MIF upregulates MMP-1 and MMP-3 in human synovial fibroblasts and MMP-9 and MMP-13 in rat osteoblasts. ISO-1 [(S,R)-3-(4-hydroxyphenyl)-4,5-dihydro-5-isoxazole acetic acid methyl ester], an inhibitor of MIF tautomerase activity, inhibits several MIF activities.

Methods: Interface (IFT) and pseudocapsular (PCT) tissues, and pseudosynovial fluids were obtained from patients subjected to revision of joint arthroplasty, MIF, MMP-1, MMP-13 and TIMP-1 levels were determined by ELISA. MIF mRNA expression was ascertained by RT-PCR. For immunohistochemistry IFT sections were stained with a biotinylated polyclonal antibody against MIF.

Results: The expression of MIF was confirmed by immunohistochemistry and RT-PCR in all tissues tested. MIF was detected in all tissue extracts (40–65 ng/g wet weight) and fluids (1–25 ng/ml). Tissue specimens were cultured in the absence or the presence of ISO-1 (100 μ M) in serum-free medium and after 72 h ISO-1 upregulated TIMP-1 production without concurrent upregulation of MMP-1 and MMP-13.

Conclusion: Since MMPs play a significant role in the loosening mechanism and TIMP-1 is their endogenous inhibitor, it is concluded that MIF might be involved in aseptic loosening.

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D1-016P

Expression of osteopontin at the initial stage of embryonic stem cells differentiation correlates with pattern of expression of adherens junction proteins E-cadherin and desmocollin 1 in pluripotent populations

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Expression and cellular localization analyses of the specific transcription factors, cell matrix and adhesion proteins are essential for understanding mechanisms of self-renewal and differentiation of pluripotent cells. Pluripotent state of embryonic stem cells (ES cells) and embryonal teratocarcinoma cells (EC cells) is maintained by specific transcription factors Oct 4, Nanog, however, it remains unclear, which extracellular matrix and cell adhesion proteins are involved in regulation of pluripotency. The extracellular matrix glycoprophosphoprotein expression of osteopontin was

examined in undifferentiated mouse R1 ES cells, embryoid bodies, differentiated embryoid bodies (5–7 days) and F9 EC cells and compared with expression patterns of adherens junction proteins E-cadherin, desmocollin 1 and transcription factor Oct 4 using immunofluorescence analysis. Expression of osteopontin was found exclusively in pluripotent cell populations: colonies, inner cells of embryoid bodies and clusters of undifferentiated F9 cells. Similar patterns of expression were revealed for E-cadherin, desmocollin1 and Oct 4 and the expression of studied proteins was decreased at the initial steps of embryonic stem cells differentiation. These results indicate that expression of osteopontin, E-cadherin and desmocollin1 as well as Oct 4 is associated with pluripotent phenotype during early differentiation of ES and EC cells. Interaction between osteopontin and different classes of integrins and assembly homophilic protein junction formed by cadherins are required for maintenance of integrity of pluripotent populations.

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D1-017P

Development of an on-line microdialysis-AAS system for separating and monitoring copper and copper-binding proteins in extracellular matrix

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Copper is an essential element for human being and is transported mainly by ceruloplasmin and albumin in the blood. An on-line microdialysis sampling technique coupled with flow injection atomic absorption spectrometry (AAS) method has been developed for separating and monitoring copper and copper-binding proteins in the extracellular matrix of blood. Microdialysates perfused through microdialysis probes were collected with a sample loop of an on-line injection valve and directly introduced into AAS by a flow injection system. Ultrapure saline solution was used as the perfusion solution at a flow rate of 1 gl/min through the microdialysis probe. The microdialysate and chemical modifier were on-line mixed at a micro-Tee and loaded into a sample loop. Precision (CV, %), based on the intra-assay ($n = 5$) and interassay ($n = 5$), were found to be $<10\%$. The *in vitro* recoveries (spiking standard solution in the microdialysate) ranged from 90.8 to 100.6% were obtained. The proposed on-line microdialysis-AAS system permitted the dynamic and continuous *in vivo* separating and monitoring of diffusible copper and copper-binding proteins in the extracellular matrix of blood of living animals after administrated with copper sulfate. Additionally, using the proposed method, diffusible copper and copper-carrier protein in the blood of patients with breast cancer was examined.

D1-018P

Interactions of follistatin with heparin and heparin analogues

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Follistatin is a secreted glycoprotein that functions as an inhibitor of TGF-beta family growth factor activin. It is found in

several forms, created by combination of alternative splicing of the last exon and proteolytic cleavage. The shortest form of follistatin (FS-288) lacks an acidic tail encoded by the alternatively spliced exon, and is able to bind to heparan sulphates, and is found predominately as immobilized on cell surface. We have recently solved the three-dimensional structure of this domain in complex with sucrose octasulphate, and identified the major epitope for heparin binding. To understand the interactions between follistatin and heparin in more detail, we have studied the binding of the first Fs domain (Fs1) of follistatin to heparin and heparin analogues using non-denaturing gel electrophoresis with fluorescently labelled heparins, isothermal titration calorimetry and gel filtration combined with non-dissociative mass spectrometry. These studies show that Fs1 can bind a hexasaccharide of heparin with high affinity and with 1:1 molar ratio. Longer heparin fragments typically bind two Fs1 domains, as indicated by shift in elution position from gel filtration column and mass spectrometric analysis. It is not known whether 2:1 stoichiometry is indicative of follistatin–heparan sulphate interactions *in vivo*, but we speculate that it is possibly a mechanism by which two follistatins can facilitate their interactions with a dimeric activin molecule.

D1-019P

Super-co-induction of lipase and 15 kDa protein from several *Pseudomonas* strains by fatty alcohols

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Thermo-stable and high stereo-selective lipase and esterase have recently attracted much attention from viewpoints of industrial usage. In the course of our studies searching for efficient inducers for lipase, among various additives, we have found that fatty alcohols act as the most effective “super-inducers” for induction of lipase production by several *Pseudomonas* and other *Pseudomonas*-like bacteria. Recently, several strains that show more effective lipase induction by fatty alcohols were isolated. The addition of stearyl alcohol or palmityl alcohol brought about more than several hundred-fold enhancement of the lipase activity compared to the case with no additive. This means ca. 30- to 60-fold enhancement of lipase activity compared with olive oil grown case. “Gram per litter” lipase production has been capable. We also found that when several *Pseudomonas* strains were grown on fatty alcohols, not only lipases but a large amount of an extracellular 15 kDa protein was strongly induced. This induction of the 15 kDa protein seems to be “simultaneous” with that of lipases. The 15 kDa protein has -PXXPXXE- sequence and seems to be an activator-protein. Productions of those two proteins are possibly under the same regulation system. Over-expressed lipases, deduced from the cloned nucleotide sequences, have high homology among other *Pseudomonas* lipases including lactonizing lipase. Lactones are widely distributed in nature, and have been synthesized from the corresponding omega-hydroxy fatty acids (one of cutin monomers), inducer of cutinase in a fungal system, and above strains possess lactone-specific esterase. Although palmitic acid is a certain level of lipase inducer, omega-hydroxy palmitic acid is all but lost on lipase production.

D1-020P**Expression pattern of extracellular matrix proteins characterize distinct stages of cell differentiation during antler development**É. Korpos¹, A. Molnár², P. Papp², I. Kiss¹, L. Orosz¹ and F. Deák¹¹*Institute of Biochemistry, Biological Research Center, Szeged, Hungary,* ²*Institute of Genetics, Agricultural Biotechnology Center, Gödöllő, Hungary,* ³*Department of Genetics, Eötvös Loránd University, Budapest, Hungary.* E-mail: korpos@brc.hu

The regeneration process of deer antlers is uniquely intense and complex: chondrogenic and intramembranous ossification takes place simultaneously. Cell differentiation in the developing antler of red deer, *Cervus elaphus*, was characterized with extracellular matrix markers. Expression of the four matrilin genes was monitored by immunohistochemistry and *in situ* hybridization and compared with cartilage markers collagen II and cartilage link protein, the bone component collagen I, and the endothelial basement membrane constituent laminin. The mesenchyme layer at the very tip of the velvet antler was enriched in link protein, indicating the role of hyaluronan in apical morphogenesis. Matrilin-2, a component of hard and soft connective tissue matrices, was identified here also as a marker of cells with high differentiation potential: it is expressed predominantly by mesenchyme cells, pre-chondrocytes and pre-osteoblasts. In addition to matrilin-3, expression of the other three matrilin genes was detected in osteoprogenitor cells and osteoblasts. A layer of elongated cells, which surrounded the perivascular channels, expressed all four matrilins and collagen I. As a consequence, all four matrilins, including matrilin-1, previously detected in the skeleton only in cartilage, were found associated to collagen I-rich structures in a thin layer bordering the hypertrophic chondrocyte columns. Cells with similar morphology and expression pattern were identified in the periosteum. Altogether all cell types of the chondrogenic and osteogenic lineage, which expressed the four matrilins were in separate studies positive for parathyroid hormone-related peptide and its receptor.

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D1-021P**Targeting breast cancer at the gene expression level of the effective matrix macromolecules proteoglycans, MMPs and TIMPs**

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Modified gene expression of extracellular matrix molecules (ECM), structural alteration as well as degradation of ECM by neoplastic cells is a prerequisite for invasion and the formation of tumour metastases. Differential synthesis of proteoglycans (PGs) in stroma and epithelial cells has been related with breast tumorigenesis and proliferation, differentiation and the invasive properties of human epithelial breast cancer. Tumour cells can also release metalloproteinases (MMPs) that degrade the matrix macromolecules and enhance invasion through tissue barriers, blood vessel and lymph channel wall. In order to examine whether gene expression of PGs and MMPs is related with breast cancer and invasive potential, we performed *in vitro* studies on a panel of epithelial breast cancer cell lines (oestrogen

receptor-positive and -negative of low and high invasive-ness). The obtained results clearly showed that breast cancer is associated with significant changes in gene expression of secreted PGs (decorin, biglycan and versican) as well as cell surface PGs (glypican, syndecans, thrombomodulin and CD44). Significant changes in gene expression of MMPs and TIMPs were also found. It is worth noticing that the expression of certain types of MMPs can be related with the invasive properties of the epithelial breast cancer cells. Studies to elucidate whether the modified gene expression of PGs, MMPs and TIMPs are associated with certain molecular targets and their respective signalling pathways, using specific tyrosine kinase inhibitors, such as STI571 and genistein, as well as the specific P450 aromatase inhibitor letrozole, showed that both tyrosine kinase pathways and oestrogen receptors are of crucial importance for cancer cell growth and modified gene expression of matrix effectors.

D1-022P**Effects of zoledronic acid on serum levels of MMPs and bone remodeling markers in breast cancer patients with bone metastases**I. Kanakis¹, M. Nikolaou², C. Kiamouris³, D. Pectasides⁴ and N. Karamanos¹¹*Laboratory of Biochemistry, Department of Chemistry, University of Patras, Patras, Greece,* ²*Department of Medicinal Oncology, Metaxas Memorial Cancer Hospital, Piraeus, Greece,* ³*Department of Epidemiology, University of Athens, Athens, Greece,* ⁴*Second Propaedeutic Department of Internal Medicine, Athens University Medical School, Attikon Unive, University of Athens, Athens, Greece.* E-mail: jkanakis@upatras.gr

Biochemical markers of bone turnover, bone alkaline phosphatase (BAP), and N-telopeptide of type I collagen (NTx) have been developed to assess metastatic bone disease. Matrix metalloproteinases-2 and -9 are proteolytic enzymes which play key role in metastatic process. Zoledronic acid (ZA) is a potent bisphosphonate for the treatment of patients with bone disease. The aim of this study was to study the effect of ZA on serum levels of these specific markers. Forty-six breast cancer patients were enrolled in this study (24 with bone metastases under ZA treatment and 22 with extraskeletal metastases). Serum levels of markers were measured by ELISA, at baseline and every 1 month thereafter. At baseline, MMP-2, BAP, and NTx were elevated in patients with bone metastases compared with the other group (31.8 ± 8.4 ng/ml vs. 29.2 ± 6.2 ng/ml for MMP-2, 57.6 ± 35.8 U/l vs. 42.2 ± 33.9 U/l for BAP, 28.1 ± 23.5 nM BCE vs. 22.1 ± 10.8 nM BCE for NTx), but for MMP-9 the result was opposite (6.8 ± 1.7 ng/ml vs. 10.6 ± 2.4 ng/ml), suggesting that MMP-9 is also involved in metastasis to other sites except bone. In follow up, MMP-2 and BAP showed decreased but not statistically significant changes (29.5 ± 8.1 ng/ml and 28.0 ± 9.9 ng/ml, 7.3% and 12.0% decrease, 35.7 ± 44.8 U/l and 52.8 ± 42.0 U/l, 38.2% and 8.3% decrease for BAP), while MMP-9 remained at the same levels. NTx showed excellent response to ZA administration (19.0 ± 13.7 nM BCE and 13.9 ± 6.9 nM BCE, 32.3% and 50.5% decrease, *P* < 0.005). NTx appears to be a useful marker in assessing bone metastatic status in patients with breastbone metastases. BAP levels did not show a significant change and only MMP-2 can be correlated with bone disease. Although changes in MMP-2 levels were not significant, there is an obvious tension of decrease during ZA treatment.

D1-023P**Arthritis *in vitro*: different activation of matrix metalloproteinase enzymes by traumatic or septic arthritis inductors**E. Karakine¹, D. Barnewitz¹, I. Ponomarev¹, A. Donchenko² and I. Wilke¹¹Forschungszentrum für Medizintechnik und Biotechnologie e.V., Bad Langensalza, Thüringen Germany, ²Institute of Veterinary Practice, Novosibirsk, Russian Federation. E-mail: ekarakine@fzmb.de

Matrix metalloproteinase (MMP) family plays the key role in cartilage extracellular matrix degradation and in the development of joint osteoarthritic (OA) diseases. We have shown previously that both normal (N) equine cartilage cells and N chondrocyte cultivated cells (CCC) display only MMP-2, but the extracts from OA cartilage contains from 2–3 (chips) up to 14 (traumatic OA) different MMPs including MMP-53 kDa (K), MMP-2, MMP-85K, MMP-9, etc. The aim of this study was to reveal special media factors to create *in vitro* models for traumatic (T) and septic (S) OA. We have found T- and S-factors for the induction of T-OA and S-OA in equine CCC, respectively, using MMP-test (reverse zymography) for the diagnosis. After *in vitro* T-OA induction, CCC themselves contain MMP-2 only; the secretion MMP pattern of CCC gives rise up to 17 different fractions including all of 14 MMPs of OA traumatic cartilage *in situ*. This T-OA MMP pattern may regress to a normal pattern reflecting the elimination of T-factor from the culture media. After S-OA induction, we have also revealed a new type of pattern: S-OA CCC themselves express 7–8 of MMPs including MMP-46K, MMP-2, MMP-85K, MMP-9, etc.; their secretion MMP pattern gives rise up to 19 fractions. These S-OA CCC reactions were not reversible if no drugs were added to the culture media. The data obtained, permit to conclude that there may exist different variants of CCC reactions for different forms of “artificial” arthritis *in vitro* and that there are specific CCC MMP patterns for the reactions.

D1-024P**Truncated forms of syndecan-1 induce differentiation of hepatoma cell lines**L. Szilák¹, E. Tátraí², P. Hollósi², S. Paku² and I. Kovalszky²¹Szilák Labor, Szeged, Hungary, ²Molecular Diagnostics, Ist Institute of Pathology and Experimental Cancer Research, Semmelweis University, Budapest, Hungary. E-mail: koval@korf1.sote.hu

Syndecan-1 is a marker of differentiated epithelial phenotype. Malignant transformation disrupts the expression of the molecule. However, we have no clear idea about the significance of its altered expression in the behaviour of cancer. Our previous result showed two typical alterations in syndecan-1 expression. These are a decrease in or disappearance of expression, and irregular localization. The objective of our work was to establish an *in vitro* model to study the effect of syndecan-1 extracellular and intracellular-transmembrane domains designated as full, 77sig, and 78sig, on the behaviour of hepatoma cell lines. HepG2 and Hep3B cells were transfected with GFP-syndecan-1 constructs containing the full or truncated cDNAs. GFP fusion destroyed the activity of intracellular domain of full but not of the 77sig and 78sig constructs. Stable transfectant with the full construct required an increased amount of serum for growth, but no phenotypic alterations have been found. In contrast, cells stably expressing the 78sig truncated form started to differentiate, forming islands with hepatocyte-like cells in the case of HepG2, or losing the sarcomatous feature in the case of Hep3B. The growth rate of the transfected cell lines decreased. Intrinsic syndecan-1 protein expression was upregulated by the transfection. This went together with the

reappearance of hepatocyte-specific albumin and the asialoglycoprotein receptor. The most striking effect of the construct was the downregulation of Ets-1 transcription factor. As Ets-1 is involved in the upregulation of matrilysin expression, which cleaves syndecan-1 ectodomain, it is conceivable that transfection with the truncated version of syndecan mimics the shedding of the molecule, activating a negative feedback on Ets-1 protein.

D1-025P**Distribution of hyalectans and small proteoglycans in cerebrum, cerebellum and brainstem of young sheep brain**

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Hyalectans are large aggregating proteoglycans (PGs) that carry mainly chondroitin sulphate (CS) side chains. Members of hyalectan family are currently versican, aggrecan, neurocan and brevican, occurring in brain with various biological functions. Brain contains also two types of small size CS or/and dermatan sulphate (DS)-containing PGs, decorin and biglycan. The aim of this study was to evaluate the localization and the content of hyalectans and small PGs in the three distinct areas of young sheep brain. Brain PGs were extracted from delipidated cerebrum (CB), cerebellum (CL) and brainstem (BS) and were analysed by biochemical methods, including immunoblotting and high-performance liquid chromatography (HPLC). Moreover, brain sections were examined immunohistochemically using a panel of antibodies directed against versican, aggrecan, brevican, neurocan, decorin and biglycan. The relative amount of individual PGs varied significantly among the extracts of different areas of sheep brain. Biochemical analyses indicated that versican's content is greater in brainstem, in contrast to neurocan and decorin that is greater in cerebellum, while aggrecan is greater in cerebrum and brainstem. Disaccharide analysis of CS/DS PGs from the three areas of the brain showed that 4-sulphated Delta-disaccharide (Δ di-4S) is the major component in the CS/DS chains of brain PGs. The ratio of Δ di-4S to 6-sulphated Delta-disaccharide (Δ di-6S) appeared to be reduced in BS compared with CB and CL. The obtained data clearly demonstrate that the GalGAGs disaccharide content significantly varies in the three areas of the brain. The different composition of PGs (hyalectans, decorin, biglycan) in the three distinct areas of young sheep brain is crucial for the biological role of each molecule in the development of the nervous system.

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D1-026P**Disintegration of collagen metabolism between the various organs as a possible reason for their pathology**

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Pathological changes occurring in the body under the effect of stress can be caused by the disintegration of the metabolism in the tissues of various organs at the level of the so-called “integration links”. By this term we mean a logically assumed and mathematically proved community of metabolic characteristics at the inter-organ and inter-tissue level. In the graphic expression this is represented by the area inside which the adaptation mech-

animals of various systems cross. Disturbance of “integration link” at the collagen metabolism level is shown by an “experimental osteochondrosis” model (dystrophically destructive process in the spine proved histologically). It was provoked by the separation of an intervertebral disk from the vertebral body by two vertebral segments after a lumbar spine operation (white male rats of 12 month age). The tissues of intervertebral disks, bodies of vertebrae, femoral bones, myocardium and aorta were taken for the analysis. The ¹⁴C-proline radioactive tracer was injected intraperitoneally 24 h before the animal decapitation. The decrease in the amount of the total proline has been found in all the observed tissues, the intensity the ¹⁴C-proline absorption has increased in all the tissues except the vascular one, but the hydroxyproline concentration has changed selectively: it increased in femoral and vertebral tissues and decreased in heart, vessels, intervertebral disks. It is possible to suppose that in the osteal tissues it is directed to the collagen synthesis. At the same time it is used for other purposes in the intervertebral disks, cardiac and vascular tissues, for example, for energetical ones. A competition for the proline is possible between the tissues. It may be basis for combination of various organ pathology.

D1-027P

Effect of vasoactive peptides on adhesion and chemotaxis elicited by extracellular matrix protein sequences

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Vasoactive peptides are considered to be regulatory factors in physiological disorders. The interaction of cells with extracellular matrix (ECM) is important in cell physiological processes. Constitutive sequence RGD of ECM proteins is recognized by the integrins. Obstatin is $\alpha\beta 1$ -specific disintegrin, have the KTS integrin-binding motif. A non-integrin receptor for ECM components is elastin-binding protein (EBP), a specific binding site of VGVAPG motives of the elastin.

Objectives: To investigate (i) the capability of RGD, KTS and VGVAPG peptides to induce *in vitro* cell adhesion and chemotaxis; (ii) the relation of adhesion and chemotaxis in cells being on distinct levels of dedifferentiation; (iii) the influence of vasoactive peptides: angiotensin-II (ATII), endothelin-1 (ET-1), apelin-13 (Ap13), on the above-mentioned parameters.

Methods: Applied model-cells: THP-1 and J-774 monocytes, and MRC-5 fibroblasts. The chemotactic ability was determined in NeuroProbe® chamber. The cell adhesion assays were done with peptide-coated immunoplates.

Results: (i) All three ECM peptides elicit a concentration-dependent, adhesion in J-774 and MRC-5 cells, while adhesion of the most dedifferentiated THP-1 monocytes was induced slightly by the specific KTS peptide. (ii) VGVAPG and RGD peptides, have a strong chemoattractant effect on MRC-5 and J-774, at higher concentrations (10^{-6} M), while THP-1 cells was sensitive to the KTS peptide. (iii) Cell pre-treatments with vasoactive peptides perturb their responsiveness with diverse, vasoactive peptide-specific outcome.

Conclusion: The investigated peptides have ligand-specific effects in different cell lines. The distinct influence of the vasoactive peptides suggests their paracrine regulatory role on cell migration.

D1-028P

Regulation of MMP-13 by nitric oxide and association with caveolin-1

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Matrix metalloproteinase (MMPs) are implicated in matrix remodeling during proliferative, inflammatory and angiogenic process including wound healing, whereas VEGF is a critical cytokine involved in angiogenesis, and nitric oxide (NO) is a downstream effector. We have shown that NO induces MMP-13 expression and activity in bovine and mouse aortic endothelial cells. We have demonstrated that aortic endothelial cells from eNOS null mice present delayed migration and a significant decrease of MMP-13 expression. We also demonstrated that MMP-13 was localized to caveolae, forming a complex with caveolin-1. Caveolins are structural proteins used by cells to form caveolae, involved in normal signal transduction pathways and in the pathogenesis of several pathological entities. In an effort to determine the precise mechanism by which MMP-13 interacts with caveolin-1, we identified the caveolin-1 Scaffolding domain as a docking region to which MMP-13 is bound, as detected by incubation with a synthetic peptide comprising the caveolin-1 scaffolding domain. Stimulation with NO disrupted this complex and significantly increased extracellular MMP-13 abundance, leading to collagen breakdown. Taken together these results indicate that MMP-13 mediates nitric oxide activation of endothelial cell migration.

D1-029P

Decorin transfection in breast cancer cells induces proteomic modulation and downregulation of matrix proteases

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The progression of cancer is associated with multiple gene alterations and/or defective gene expressions. During the invasive growth neoplastic cells enter in dynamic contact with several components of the extracellular matrix that may influence gene expression and induce phenotypic modulation of neoplastic cells. Among these environmental factors, decorin, a representative member of the small leucine-rich proteoglycan family, occupies a central role because of its ability to interact with collagens and cellular receptors and to modulate biological activities. To test the effects of ectopic decorin expression in neoplastic cells we performed a comparative study of proteome and matrix proteases of decorin-transfected 8701-BC clones vs. the parental cell line, applying 2D-IPG, zymography, Western blot and RT-PCR methodologies. Our preliminary results showed that the protein complement expressed by cells following transfection undergoes significant modifications. Protein modulation involves some cytoskeletal proteins, metabolic enzymes and chaperonins. Cell morphology assays show remarkable cell surface modifications of

transfected clones. Since cytoskeleton, besides its role in maintaining cell polarity, is also involved in signal transduction, its modification in the transfected clones is probably associated with complex responses induced by ectopic decorin. In addition, transfected clones display a significant reduction of the levels of matrix proteases released into the media culture, which correlates with a downregulation of the transcription of the corresponding genes. These results provide additional insights into the reported effect of decorin in neoplastic cell behaviour.

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D1-030P

Distribution of hyaluronan and hyaluronan-associated proteins in the spinal cord of chicken embryos

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Hyaluronan is a peculiar non-sulfated glycosaminoglycan that is generally present in the extracellular matrix (ECM). The interaction of HA with HA-binding ECM molecules or CD44 and RHAMM cell surface receptors regulates many aspects of cell behavior including cell migration, differentiation and cell adhesion to another cell or ECM. Based on our studies, we speculate whether the HA acts as an autocrine or paracrine regulator through hyaluronan receptors (CD44 and RHAMM) or it is involved in a different signaling pathway. By using a specific HA binding probe derived from aggrecan we found strong HA signal in the intermediate zone in the cross-sections of chicken embryos from the age of 23 stages according to Hamburger and Hamilton (HH) while the other part of the spinal cord showed a moderate (marginal zone) or loss of (germinative zone, floor plate) HA signal. We could not find any CD44 expression in the spinal cord of the chicken embryos until they reach the HH36 stage. By using RT-PCR we have demonstrated that HA found in the spinal cord of chicken embryos is produced by hyaluronan synthase 2 (has2). HA reaction in the intermediate zone in the developing spinal cord of chicken embryos may indicate a permissive role of the HA molecule during the early stage of neuronal development. It is known that HA is produced by has2 requires upstream Rac1 small GTPase which is thought to have an effect on actin polymerization during lamellipodia formation through CD44 or RHAMM receptor. In our experimental model, however the involvement of CD44 can be neglected in this signaling pathway.

D1-031P

Abnormalities of syndecan-1 expression in precancerous and malignant lesions of the oral cavity and uterine cervix

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Syndecan-1, a transmembrane proteoglycan may exert antiproliferative effects, but may also promote cell growth by binding various

growth factors. Malignant epithelial cells often downregulate their own syndecan-1 production, whereas they are capable of inducing an aberrant syndecan-1 expression in stromal fibroid cells. Immunohistochemical analysis performed on 35 oral leukoplakias, 51 invasive oral squamous cell cancers, and 39 cervical carcinomas revealed one or both of the above alterations concerning syndecan-1 expression. A decrease in syndecan-1 expression compared with normal epithelium could occasionally be detected as early as in leukoplakias, representing pre-malignant oral lesions. Syndecan-1 expression of tumor cells was decreased or even completely lost in 45 of 51 oral carcinomas and 37 of 39 cervical carcinomas. Furthermore, tumor-induced stromal syndecan-1 immunoreaction appeared in 19 of 51 oral tumors. In the case of oral cancers, but not of the cervical carcinomas, the probability of postoperative progression showed some dependence on the degree of decrease in tumor cell syndecan-1 levels; still the correlation was not statistically significant. Based on recurrence and overall survival data, stromal syndecan-1 expression in primary oral cancers appears to be a more reliable factor of adverse prognosis; however, the question whether the presence and extent of stromal syndecan-1 expression can be considered real risk factors of postoperative progression in oral malignancies requires further investigation.

D1-032P

Angiotensin II induces a tyrosine kinase-dependent increase in metalloproteinase activity in endothelial cells

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Matrix metalloproteinase 2 (MMP 2) comprise a subfamily of metalloproteinases (MMPs) capable of digesting basement membrane proteins. High expression of MMPs has been reported in various pathologic conditions associated with angiogenesis and tumor invasion. Angiotensin II (Ang II), a bioactive peptide of renin-angiotensin system, regulates numerous physiologic responses, such as salt and water balance, blood pressure, vascular tone, and vascular remodeling, and it is also involved in the pathogenesis of a number of cardiovascular diseases. In the present study, we demonstrated that Ang II provokes a dose-dependent increase in MMP 2 activity in lysates and conditioned media of human umbilical vein endothelial cells (HUVEC). Pretreatment of cells with 10 µM PP2, a selective inhibitor of Src family tyrosine kinase, or 10 µM U73122, a specific inhibitor of phospholipase C (PLC), markedly decreased Ang II-induced MMP 2 activity. Nevertheless, pretreatment of HUVEC with 0.5 µM wortmannin, a selective inhibitor of phosphoinositide 3-kinase (PI3K), does not modify Ang II-induced MMP 2 activity. These results seem to indicate that Ang II modulates the synthesis and secretion of MMP 2 from endothelial cells through a PLC and Src tyrosine kinase-dependent pathway, and suggest that PI3K is not involved in Ang II-induced MMP 2 regulation.

D1-033P

Structural determinant on self-assembly of modeled human elastin polypeptides

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Elastin is an extracellular matrix protein found in large blood vessels, lung, ligaments, and skin, imparting the physical properties

of extensibility and elastic recoil to these tissues. To achieve the required structural durability of the elastic matrix, the elastin monomer, tropoelastin, undergoes ordered assembly into a covalently cross-linked, fibrillar polymeric structure. Human tropoelastin consists of 34 exons coding for alternating hydrophobic and cross-linking domains. Using a series of well-defined recombinant polypeptides modeled after human elastin sequences and domains, we are investigating the mechanism of hydrophobically driven self-assembly of the elastin polymer. We have shown that both sequence and context of hydrophobic domains, as well as polypeptide chain flexibility affect the propensity of these polypeptides for self-assembly. Here, we report that the sequence and structure of cross-linking domains also have significant effects on assembly of elastin polypeptides. Removing a putative flexible hinge region in the cross-linking domains substantially increased the α -helical content and strongly promoted self-aggregation. While trifluoroethanol (TFE) promoted and urea inhibited self-assembly of these polypeptides, these effects were not related to altered α -helicity of the polypeptides. These results suggest that, while increased α -helicity also favors this process, stabilization of β -turn structures in the more flexible hydrophobic domains are a major determinant of self-assembly of elastin-like polypeptides. Such simple elastin polypeptide models can provide an important tool for understanding the relationships between sequence, structure, and polymeric self-assembly of elastin.

D1-034P
Functional analysis of the regulatory regions of the matrilin-1 gene in transgenic mice reveals modular arrangement of tissue-specific control elements

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Matrilin-1 functions in the organization of the extracellular matrix. It is secreted primarily by chondrocytes in a characteristic pattern during skeletogenesis. As a first step to define the tissue- and site-specific regulatory regions of the chicken matrilin-1 gene *in vivo*, we generated transgenic mice harboring various promoter and intronic fragments fused to the LacZ reporter gene. Histologic analysis of the transgene expression pattern revealed specific X-gal staining in most primordial elements of endochondral bones of transgenic mouse lines carrying either the long promoter or the intronic fragment with a short promoter. The cartilage-specific activity of the latter transgene, however, was accompanied with variable ectopic expression pattern in neural and other tissues depending on the site of integration. The presence of both promoter upstream and intronic elements was necessary for the high-level transgene activity in all chondrogenic tissues and for the extraskelatal transgene expression pattern resembling the most to that of the chicken matrilin-1 gene. The activity of the transgenes was restricted to the columnar proliferating and prehypertrophic chondrocytes visualized by BrdU incorporation and distribution of phosphorylated Sox9. DNA elements between -2011 and -338 also mediated ectopic LacZ expression in cells of neural crest origin. These results suggest that an interplay of modularly arranged cartilage- and neural crest-specific DNA elements control the expression of the matrilin-1 gene. The dispersal of cartilage-specific elements in the promoter upstream and intronic regions shows similarity to the transcriptional regulation of the Col1a2 gene.

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D1-035P
Normal and cancerous osteoblastic cells display distinct differences in the expression of matrix chondroitin sulphate proteoglycans

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Pathogenesis of osteosarcoma, implicates qualitative and quantitative changes in proteoglycans (PGs) component of extracellular matrix. Aim of the present study was: (i) to compare the expression profile of chondroitin sulphate proteoglycans (CSPGs) of the extracellular matrix (ECM), between normal and cancerous osteoblastic cells and (ii) to study the differential effect of TGF α , bFGF and PDGF-BB on the biosynthetic pathways of CSPGs. We utilized primary human osteoblastic cells isolated from a human periodontal ligament and the osteosarcoma cell line MG63. PGs were isolated from cell culture supernatants by ion-exchange chromatography and identified using Western blotting. Analysis of the mRNA content was performed using real-time PCR for versican isoforms, aggrecan, decorin, biglycan, perlecan and hyalouronan synthases HAS1, HAS2 and HAS3. In addition, we performed metabolic labelling and subsequently HPLC analysis and quantification of the glycosaminoglycans (GAG) secreted components. Western blotting demonstrated increased basal level of decorin and very low levels of versican and aggrecan in the MG63 cells. Conversely, we observed low basal levels of decorin and high levels of versican and aggrecan in the normal osteoblastic cells. Treatment with growth factors showed minor effects on the CSPGs biosynthesis in normal cells, whereas the effect was more pronounced in the cancerous cells mainly on the biosynthesis of decorin with a significant stimulation. Versican 0 and V1 transcripts were extremely low in MG63 cells when compared with normal whereas TGF α treatment completely restored their expression. MG63 cells demonstrated eight times less HAS2 mRNA expression than the normal osteoblasts, which was completely reversed by the treatment with TGF α . The significant differences in the biosynthetic pathway of CSPGs between cancerous and normal osteoblastic cells may suggest an important role in these PGs in osteosarcoma development.

D1-036P
Spatio-temporal induction of matrix metalloproteinases in a functionally reversible light damage of the rat eye

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Matrix metalloproteinases (MMPs) are a family of proteolytic enzymes that play crucial role in maintenance and remodeling of tissue architecture-digesting extracellular matrix proteins and membrane adhesion molecules. Upregulation of MMPs at transcriptional and protein level have been demonstrated in different

over-stimulation paradigms in the nervous system, such as kainate epilepsy model and ischemia. In these situations, high intensity stimulation-induced tissue remodeling results in irreversible functional changes of the affected area. Because of neuronal plasticity, the size of the area in which MMP activation occur highly influences the changes in tissue remodeling derived alterations in physiologic functions. Consequently, not only the activation by itself but the spread of MMP activity is a matter of interest. To find out whether MMPs can or cannot be activated by an intense but physiologic stimulus that induces only temporary changes in physiologic functions, we used a light-induced, reversible retinopathy model on rats. Temporal distribution of MMP-2 and -9 protein was analyzed by gelatin zymography from the retina and the surrounding tissues of the eye. Changes in MMP-2 and -9 mRNA level was investigated by RT-PCR technique. To characterize the physiologic activity of the retina, electroretinogram (ERG) was detected from freely moving animals. We have observed rise of the protein and the mRNA level of MMP-9 and with a lesser degree, MMP-2 after light damage in all layers of the eye but the temporal patterns were different in various areas. The b-wave amplitude of the ERG disappeared after light exposure, but returned to 85% of the control value by day 7 after illumination. We can conclude that MMPs can be activated by intensive light exposure. We also demonstrated that MMP activation could spread to the neighboring, non-neuronal tissues of the eyeball via messages activating MMP mRNA transcription, but not by diffusion or excitation spreading.

D1-037P

Characterization of bioconjugates of protein and polysaccharide from bifidobacteria

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Analysis of *Bifidobacterium adolescentis* 94-BIM cells with electron microscope revealed the existence of extracellular fibrillar structures bridging the cells. Bioconjugates of protein and polysaccharide was found to be bound to the outer surface of the cell wall. However, at the late stages of *B. adolescentis* 94-BIM cultivation (48–72 h of growth), it also occurred in the culture liquid, which can be explained by the expansion of the outer material of the cell wall. Bioconjugates isolated from the cell surface and culture liquid contained proteins and carbohydrates in a ratio of 1:1 and from 1:4 to 1:5, respectively. Bioconjugates exerted a concentration-dependent bifidogenic effect-stimulated growth, acidogenesis, accumulation of extracellular proteins and enzymes, and sugar utilization in bifidobacterial cells in synthetic medium. Bioconjugates also promoted the rehabilitation of bifidobacterial anabiotic forms. The polysaccharide type was obtained from cells by sonication, ethanol precipitation and purified by ion-exchange chromatography on DEAE-Sephadex A-25. This polysaccharide was produced in different culture media, including synthetic medium supplemented with lactose. Results of methylation analysis revealed the presence of terminal, 4-substituted and 4,6-disubstituted glucose residues. Immune rabbit serum against whole bacterial cells reacted predominantly with homologous polysaccharide. The results of chemical study indicate the structural heterogeneity of the surface polysaccharide.

D1-038P

Altered expression of tight junction proteins in HCC and metastasis liver tumours

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Cell adhesion has an important role in tumour progression. Tight junction (TJ) proteins have already been found implicated in carcinogenesis. Expression of claudins, occludin, junctional adhesion molecule (JAM)-1, -2, -3 and zonula occludens (ZO)-1, -2, -3 was analysed in 15 human hepatocellular carcinoma (HCC) and 15 liver metastasis of colon cancer to study TJ in liver malignancies. Gene expression levels were measured by real-time PCR, protein localization was determined by immunohistochemistry comparing tumours to surrounding parenchyma and to normal liver samples (seven). ZO-2, JAM-2 and occludin mRNAs were significantly downregulated in HCC compared with normal liver (15.3x, 5.9x and 8.2x) and to surrounding tissues (3.4x, 3.2x and 2.2x). In metastasis claudin-3 and -4 were significantly upregulated (6.4; 12.7x), while ZO-2, JAM-2 and occludin were downregulated (9.6x, 18.6x, 12.1x) with respect to normal liver. Immunohistochemistry basically supported RNA expression data. Claudin-3 and -4 staining were very strong in metastasis, while only scattered weak in HCC. TJ proteins were generally weakly expressed on hepatocytes, while strongly on bile canaliculi and arterioles in normal liver, however. HCC and metastasis show different characteristics of RNA and protein expression of TJ components, which might refer to unique biological features of these tumours and might be used for differential diagnosis. Further, claudin-3 and -4 proteins might serve as targets of adjuvant therapy.

D1-039P

Polyhydroxyalkanoates degrading extracellular hydrolase-like activity by *T. thermophilus*

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Many bacteria produce polyhydroxyalkanoates (PHAs) as intracellular carbon and energy storage materials. PHAs have attracted commercial biotechnological interest because of their biodegradability and biocompatibility. The PHA-degrading micro-organisms secrete extracellular PHA depolymerases, which degrade PHA and utilize the products as nutrients. Only a few studies appeared for thermophilic PHA-degrading bacteria and thermostable PHA depolymerases. Elevated temperatures improve dramatically the bioavailability and solubility of organic polymeric compounds and biodegradable environmental pollutants allowing efficient bioremediation. *T. thermophilus* cultures were checked for its potential in the secretion of esterase activities when the micro-organism grows under accumulating conditions, in the absence and presence of exogenous PHB as inducer. Cells were grown in MSM containing 1.5% (w/v) sodium gluconate, 0.25% (w/v) PHB and PHB plus sodium gluconate. Cell growth was monitored, by measuring the absorbance at 600 nm. In each case, the extracellular PHA depolymerase activity was followed as *p*-nitrophenyl butyrate (PNPB) esterase activity at 65 °C during growth. An extracellular hydrolytic activity responsible to hydrolyse these substrates is secreted into the culture media as the cells grow and reached its maximum 11, 9, and 12 U/l respectively, after 72 h of culture growth. PHB depolymerase activity was also assayed spectrometrically using poly(3HB) as substrate by measuring the decrease in turbidity due to insol-

uble PHB at 650 nm over a period of at least 60 min. The PHB depolymerization exhibited a lag-phase and then proceeded linearly for about 45 min. PHB concentrations higher than 30 µg/ml inhibited enzyme activity. The poly(3HB) depolymerase of *T. thermophilus* was purified by an affinity method based on a selective binding to poly(3HB)-coated silica beads. The enzyme was purified almost to homogeneity by one step.

D1-040P

Endoglin- haploinsufficiency modifies biological properties of murine endothelial cells

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Endoglin (CD105) is a transforming growth factor-β (TGF-β) co-receptor expressed mainly on endothelial cells and involved in vascular remodelling and angiogenesis. Mutations in the coding region of CD105 gene are associated with hereditary haemorrhagic telangiectasia type 1 (HHT1), a dominantly inherited vascular disorder characterized by multisystemic vascular dysplasia. The aim of the present study was to evaluate the role of endoglin on cell proliferation, migration and extracellular matrix synthesis in endothelial cells from endoglin heterozygous (*Eng*^{+/-}) mice. We generated primary cultures of mice aortic endothelial cells (MAEC) from endothelial sprouts derived from aorta rings from *Eng*^{+/-} C57BL/6 and wild-type mice. Western blot analysis revealed that CD105 expression was reduced by 50% in *Eng*^{+/-} MAEC. The rate of cell growth was faster in control than in *Eng*^{+/-} MAEC. Furthermore, flow cytometry analysis demonstrated that endoglin haploinsufficiency-induced cell cycle arrest at the G0/G1 phases. Migration inhibition was observed in *Eng*^{+/-} cells measured by both, a multichannel wounding and a transwell system. Collagen and fibronectin synthesis, assessed by [³H]-proline incorporation and Western blot respectively, was higher in *Eng*^{+/-} MAEC than in control cells. Connective tissue growth factor (CTGF) expression was 10-fold higher in *Eng*^{+/-} MAEC than in control cells. Treatment with TGF-β1 increased extracellular matrix in MAEC, but this increment was higher in control than in *Eng*^{+/-} MAEC. These data reveal the importance of endoglin in modulating proliferation, migration and extracellular matrix synthesis in endothelial cells. The progression in our understanding on the functional role of endoglin might have important relevance in the regulation of angiogenesis processes.

D1-041P

High- and low-molecular adenosine deaminase 1

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Adenosine deaminase (ADA; EC 3.5.4.4) deaminates (deoxy)adenosine to (deoxy)inosine. ADA is widely distributed in mammalian tissues with the highest activity in lymphoid tissues. Its role is critical in proliferation, maturation and function of lymphoid cells. Three molecular forms of human ADA are known:

small and large isoforms of isoenzyme ADA1 and isoenzyme ADA2. To investigate the specific structure-function relationships in two isoforms of ADA1, the large form (LADA1), representing a high affinity complex between the small form (SADA1) and the cell membrane protein CD26/dipeptidyl peptidase IV (CD26-DPPIV), was isolated and purified from bovine kidney cortex, human pleural fluid and human blood serum. It was shown that LADA1 possesses both the ADA and DPPIV activities at all purification stages, and it was impossible to separate one activity from another. The catalytic parameters (K_m and V_{max}) in reaction of adenosine and 2'-deoxyadenosine deamination of this isoform and of SADA1 purified from bovine lung and spleen, were compared. The obtained values of these parameters for the two enzymatic forms differ significantly, and prove that the preferable substrate for LADA1 is more toxic 2-deoxyadenosine. Both isoforms of ADA1 have similar pH-profiles with relative broad optimum at pH range 6.5–7.5. For all ADA1 preparations, the inhibition constants, K_i , of two types of compounds, derivatives of adenosine and erithro-9-(2-hydroxy-3-nonyl) adenine (EHNA), were determined. In the reaction of adenosine deamination, 1-dEHNA and 3-dEHNA appeared more effective inhibitors for LADA1 than for SADA1.

D1-042P

Molecular approaches for expression and identification analysis in corneal epithelial membrane proteins

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Proteomics is an emerging area of health biotechnology that uses a plethora of protein analysis techniques, quantitate, annotate and rapidly survey the identity of protein. In combination with genomic technologies it promise to revolutionize biology as they are expected to reveal gene regulation events involved in disease and its progression as well as to generate potential target for drug discovery and diagnostics. Information at the level of the proteome is critical to understanding the function of cellular phenotype and its role in health and disease. It provides a new way to study cell behaviors and the mechanisms of disease. The ultimate goal is to characterize the information flow through protein pathways that interconnect the extracellular microenvironment with the control of gene transcription. The challenges formidable in deciphering the proteome is the development of analytical instrumentation combined with bioinformatics, which provide rapid, high-throughput, reproducible and sensitive, tools. The complex genomic sequences of several organisms are now available or rapidly approaching completion. One major challenge for researchers is the efficient use of this resource to help identify genes responsible for complex phenotypes. The ability to scan many genes to find allelic variants is essential for this work. Here, we will review the current status of proteomics and genomics and will highlight its potential in corneal epithelial healing mechanism using MALDI-TOF MS/MS, ESI-Q-TOF, PCR, RFLPs, DDRT-PCR, for characterization and identification of proteins and gene products involved.

D1-043P**Cartilage-specific Sox transcription factors bind to the conserved proximal promoter element of the matrilin-1 gene**I. Sinkó¹, A. Nagy¹, O. Rentsendorj¹, A. Daraba¹, E. Barta² and I. Kiss¹¹*Institute of Biochemistry, Biological Research Center of the Hungarian Academy of Sciences, Szeged, Hungary,* ²*Bioinformatics Group, Agricultural Biotechnology Center, Gödöllő, Hungary.*
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The unique feature of the matrilin-1 gene is that it is expressed in chondrocytes in a developmental stage-specific manner. When the activity of the regulatory regions were studied in transgenic mice, we found, in consistence with the expression pattern of the endogenous gene, that the long promoter alone or in combination with the intronic enhancer as well as the short promoter with the intronic enhancer restricted the transgene expression to the columnar proliferative chondroblasts and pre-hypertrophic chondrocytes of the growth plate cartilage. As all these transgenes shared the proximal promoter region between -338 and +67, we addressed the questions whether the short promoter itself harbors cartilage-specific control elements. We generated transgenic mice expressing the LacZ reporter gene under the control of the proximal promoter region of the matrilin-1 gene. Expression of the transgene was monitored by X-Gal staining in founder/G0 embryos. Hystologic analyses demonstrated relatively weak transgene activity in the developing skeletal elements of the chondrocranium, axial and appendicular skeleton with highest level of expression in the columnar proliferating chondroblasts and pre-hypertrophic chondrocytes. Computer analysis revealed a conserved PeI element containing inverted Sox motifs in the proximal promoter region. *In vitro* assays and *in vivo* foot printing indicated the interaction of the PeI element with chondrogenic transcription factors Sox9, L-Sox5, and Sox6 as well as other factors. Our data support the possible involvement of PeI in the tissue- and stage-specific regulation of the gene and its interaction with other cartilage-specific *cis*-elements.

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D1-044P**Keratan sulphate and chondroitin/keratan sulphate proteoglycans of young sheep brain: distribution of phosphacan, aggrecan, SV2 and RPTP-zeta/-beta in cerebrum, cerebellum and brainstem**E. A. Sinouris¹, V. G. Kilia¹, D. A. Theocharis² and N. Papageorgakopoulou¹¹*Laboratory of Biochemistry, Department of Chemistry, University of Patras, Patras, Greece,* ²*Laboratory of Biological Chemistry, School of Medicine, University of Patras, Patras, Greece.*
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Proteoglycans (PGs)-bearing keratan sulphate (KS) and/or KS/chondroitin sulphate (CS) chains have been characterized more recently in brain. In our previous studies it was shown the presence of KS in sheep brain, as also indicated that there are more than one type of KS-PGs, which differ for tissue localization. In this study, we examined the localization and content of KS-containing PGs (phosphacan, aggrecan, SV2 and RPTP-zeta/-beta) in cerebrum (CB), cerebellum (CL) and brainstem (BS) of young

sheep brain. CB, CL and BS sections were examined immunohistochemically with a panel of antibodies, tissue extracts were analysed by biochemical methods, including immunoblotting. KS-PGs extracted from the three delipidated parts of sheep brain (CB, CL and BS) were reacted with the antibodies (against phosphacan, aggrecan, SV2 and RPTP-zeta/-beta) in a different way. Phosphacan and aggrecan comprise the vast majority of the brain KS-PGs, in both CB and BS. The PGs (SV2 and RPTP-zeta/-beta) are distributed in all the three areas of sheep brain. Several KS forms were isolated from the three parts of the brain with sizes ranging from 4.9 to 15 kDa. The ability of the above KS forms to bind to prion protein was tested using the ELISA method. Two KS forms of CL were able to bind PrP-sen with a greater affinity than the other isolated brain KS types as also CS isolated from sheep brain.

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D1-045P**Physisorption of extracellular matrix proteins for cell cultures**N. Sgarbi¹, D. Pisignano¹, F. Di Benedetto¹, A. Aloisi², G. Nicolardi², R. Cingolani¹ and R. Rinaldi¹¹*National Nanotechnology Laboratory, Department of Innovation Engineering, University of Lecce, Lecce, Italy,* ²*Dipartimento di Scienze e Tecnologie Biologiche ed Ambientali, University of Lecce, Lecce, Italy.* E-mail: nicoletta.sgarbi@unile.it

Most of the types of cells survive only in adhesion conditions. In order to control in depth the geometry and environment of culture, novel gentle patterning techniques, named soft lithographies, allow one to tailor the chemistry and the morphology of this adhesion region, by creating cytophilic islands on cytophobic background. Generally, demanding multistep processes are reported in literature for patterning biomolecules, such as extracellular matrix proteins, able to drive cells in desired topologies. In this work, we achieved *in vitro* networks of neuroblastoma cells, growing on patterned physiological laminin-1 (Ln-1) matrices physisorbed on glass by one-step microcontact printing. In order to realize substrate for cell cultures as biological as possible and we avoided cell adhesion on the background simply by a specific culture medium composition, without surface chemistry treatments. We also investigated the influence of these patterned cultures on cell behaviour, functions and properties, and results in this field are presented and discussed.

D1-046P**Agtrin in the diseased liver: a multifunctional proteoglycan recently found in the liver may promote bile duct proliferation and tumour angiogenesis**P. Tátrai¹, E. Batmunkh², J. Dudás^{1,3}, K. Egedi¹, G. Ramadori³, Z. Schaff² and I. Kovalszky¹¹*1st Institute of Pathology and Experimental Cancer Research, Semmelweis University, Budapest, Hungary,* ²*2nd Institute of Pathology, Semmelweis University, Budapest, Hungary,* ³*Department of Gastroenterology and Endocrinology, Georg August University, Göttingen, Germany.* E-mail: tatpeter@korbl.sote.hu

Agtrin is a multifunctional, multidomain heparan sulphate proteoglycan (HSPG), originally described as a key organizer of the

neuromuscular junction. Later, agrin was found to play essential roles in various other basement membranes (BMs), and on the surface of neurones and lymphocytes as well. Here, we demonstrate for the first time that agrin is also present in vascular and biliary BMs of the healthy as well as of the diseased liver. Under physiological conditions, agrin is only found in the portal tracts in small quantities. The amount of agrin greatly increases in chronic liver diseases and in the hepatocellular carcinoma (HCC). In the cirrhotic liver, agrin is seen in the BMs of blood vessels and proliferated bile ducts within the connective tissue septa, whereas no agrin appears in the sinusoidal walls of the regenerative nodules. In the HCC, agrin is accumulated in the BMs of the tumour microvasculature. Agrin production can probably be attributed to biliary epithelial cells, and to smooth muscle α -actin-positive cells such as vascular smooth muscle cells and stromal myofibroblast-like cells. Myofibroblasts isolated from rat liver synthesize and secrete agrin. Like other HSPGs, agrin is also capable of binding growth factors (e.g. basic fibroblast growth factor); furthermore, it may activate intracellular signalling pathways via specific interactions with extracellular matrix receptors (e.g. α v-integrins). These features suggest a role for agrin in bile duct proliferation and neoangiogenic processes. Importantly, its presumed involvement in tumour vascularization may render agrin a future subject of antiangiogenic research.

D1-047P Quantitation of prion protein by a sensitive ELISA

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The infectious agent of prion diseases considers to be a protein denoted as PrP^{Sc}, rich in β -sheet conformation. PrP^{Sc} is a post-translationally modified isoform of the normal prion protein, PrP^C, a glycolipid anchored, plasma membrane protein, rich in α -helices. Several studies indicate that other molecules are involved in the transformation of PrP^C to PrP^{Sc} with glycosaminoglycans having a striking role not clear enough till now. The aim of the present study was the establishment of a sensitive ELISA method for the detection of PrP and identification of its strain. The ELISA plates were positively charged by the sequential reaction of GH and spermine, and then glycosaminoglycans were immobilized electrostatically onto the amino groups. Thereafter, 100 μ l of sample (sheep brain or tonsil homogenates) were added and incubated for 16 h at 4 °C. PrP was detected immunochemically and the type of its glycosylation was examined by lectins. Almost linear correlation was observed between the amount of recombinant PrP added to the activated wells and the signal obtained after its immunodetection. PrP^C and PrP^{Sc} in tissue homogenates were quantitated and distinguished after treatment with proteinase K. Lectin binding revealed that RCA differentially bound to normal and pathological samples. This method can be adapted as a diagnostic test for the detection of PrP^{Sc} from animals and humans affected by TSE. It can also be applied for strain typing of PrP.

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D1-048P How MMPs regulate avian endochondral ossification

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Vertebrate skeletal long bones are formed by a process called endochondral ossification (EO) [1]. In this process, the growth plate cartilage, localized at the extremities of long bones and formed by the segregation of chondrocytes at different stages of differentiation, is invaded by blood vessels and gradually replaced by bone tissue [2]. The proteolytic activity of matrix metalloproteinases (MMPs), a large family of zinc-dependent proteases, is essential for normal EO. Indeed, MMPs act not only on matrix remodeling but also on cell functions [1]. MMP9, MMP13 and MT1-MMP are highly expressed during EO [1] and previous studies have focused on their role during this process. Most of these studies are concerning to EO of mammals. It is known that the chicken growth plate has a distinctly different structure from the mammalian one: it is much wider, it contains more cells in each zone, and the blood vessels penetrate deeper into the hypertrophic zone [2]. This work aims to study the expression pattern and the role of MMPs during EO in the chicken and to compare them with those in the mammal. We have, so far, confirmed, by Western blot, the presence of MMP2 and MMP13 in the avian growth plate for 1-, 2- and 3-week-old animals, and both enzymes showed no significant changes in their expression level along these ages. It was demonstrated, by gelatin zymography that the growth plate shows high levels of MMP2 activity for 1-, 2- and 3-week-old animals. However, casein-impregnated gels revealed activity for MMP13 only at 3 weeks of age.

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D1-049P Production and characterization of a Vitronectin-insulin-like growth factor-I chimeric molecule

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Recent observations have demonstrated that insulin-like growth factors (IGFs) are able to form complexes with the extracellular matrix protein Vitronectin (VN). These complexes, generically referred to as VitroGro®, have been demonstrated to significantly enhance the proliferation and migration of various cell lines including skin and corneal epithelial cells, as well as primary cells derived from human skin and corneal tissue. These enhanced effects arise from co-activation of the IGF-binding

type-I IGF receptor as well as activation of the VN-binding α -integrins. Further studies suggest that these complexes can replace the requirement for serum in *ex vivo* expansion of cells. In order to translate the VitroGro® technology into techniques for the improved culture of cells, we have designed and expressed a synthetic chimeric molecule, consisting of appropriate domains of the VN and IGF-I, using a baculovirus-based expression system. The recombinant VN:IGF-I (rVN:IGF-I) chimera was secreted as an ~90 kDa protein into condition media of transfected SF-9 insect cells. Purification of the chimera was achieved via heparin-Sepharose chromatography along with Q-Sepharose ion-exchange chromatography. rVN:IGF-I is detectable in Western blotting using a polyclonal VN antibody. Structural and functional characteristics, assessed using cell-based assays for growth and migration, of the chimera will be presented. A functional chimera may also lead to the development of cell culture techniques and methodologies that are devoid of xenogeneic or allogeneic support systems, thus paving the way to approved tissue engineering therapeutics that incorporate *ex vivo* expanded adult stem and progenitor cells.

D1-050P

Specific binding of cobra cardiotoxins, members of the Ly-6 protein family, to integrin α Vbeta3

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Severe tissue necrosis with retarded wound healing process is a major symptom of the cobra snakebite. Cardiotoxins (CTXs) are

major components of cobra venoms and are implicated in tissue damage, but the mechanisms of the toxicity or cellular receptors are unknown. We show here that CTXs from Taiwan cobra, i.e. CTX A3, A5, and A6, specifically bound to α Vbeta3 integrin, an integrin highly expressed in wounds or inflammatory tissues, in the order of affinity CTX A5 > A3 >> A6. CHO cells overexpressing α Vbeta3 bind to CTXs in a cation-dependent manner at a higher level than mock-transfected CHO cells. Soluble α Vbeta3 specifically bound to immobilized CTXs in a manner similar to membrane-bound α Vbeta3. CTXs competed with fibrinogen gamma-chain C-terminal domain (gammaC), a known α Vbeta3 ligand, for binding to soluble α Vbeta3, suggesting that CTXs-binding site is close to or overlaps with the gammaC-binding site in α Vbeta3. Surface plasmon resonance study showed that recombinant soluble α Vbeta3 bound to CTX A5 and A3 with an apparent dissociation constant of approximately 0.6–0.7 μ M. Calf pulmonary artery endothelial cells constitutively expressing α Vbeta3 showed a binding profile similar to beta3-CHO cells and soluble α Vbeta3, suggesting that CTX binding to α Vbeta3 is potentially involved in vascular damage. These results establish that CTXs as a new family of non-RGD integrin-binding protein and shed new light on the toxicity of cobra venom.

D2 – Proteins in Development

D2-000

Nuclear envelope proteins involved in muscular dystrophies

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Muscular dystrophies (MD) are a group of neuromuscular disorders which arise because of defects in proteins whose functions contribute to maintaining the structural integrity of skeletal muscle. Patients suffer from progressive skeletal muscle weakness, as well as a variety of cardiac complaints. The vast majority of MDs are attributed to defects in proteins which form part of a multi-molecular complex in the sarcolemma, linking cytoskeletal components to the extracellular matrix. Unexpectedly, the genes responsible for Emery–Dreifuss muscular dystrophy (EDMD) are both localized to the nucleus. The X-linked gene encodes for a single-membrane spanning protein termed emerin, which is localized to the inner nuclear membrane, and the autosomal dominant gene is alternatively spliced to encode for two nuclear intermediate filament proteins lamin A and C. We have shown that emerin is part of a large macro-protein complex which cross-links the nuclear envelope to the nuclear lamina and chromatin. Components of

this complex include the nuclear lamins, nuclear actin, members of the nesprin family, transcription factors (GCL and BtF), an RNA splicing factor and BAF a DNA bridging protein. We have shown that emerin occurs in four different phosphorylated states, three of which are cell cycle-dependent. In a subset of X-EDMD patients emerin is aberrantly phosphorylated and cell cycle length is disrupted. The possible signal transduction mechanisms which give rise to this muscular dystrophy will be discussed.

D2-001

A neurodegenerative disease in *Drosophila* mutant for the tumor suppressor protein Patched

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The tumor suppressor morphogen Patched has extensive amino acid homology to the Niemann Pick C1 (NPC1) disease protein in the sterol sensing and transmembrane domains. The NPC disease is a pediatric progressive and fatal neurodegenerative disorder thought to be due to the abnormal trafficking of cholesterol in neurons. Here, we report that Ptc mutant adults develop progressive locomotor deficits and their neurons contain inclusions similar to NPC and other lysosomal storage disorders. Since feeding cholesterol to wild type flies generates inclusions in the brain but does not cause any neurodegenerative disease, the inclusions *per se* are

not responsible for the disease-state. However, feeding cholesterol to mutant flies reduces the progression of the disease. We find that in the central brain regions of the mutant brains there is a reduction in the number of synaptic connections and feeding cholesterol markedly improves the number of synaptic connections. Our results indicate that sequestration of cholesterol in the form of inclusions in the mutant brain causes cholesterol to become a limiting factor. This in turn causes a reduction in the number of synaptic connections and neurodegeneration and the manifestation of disease-state. These results are consistent with the previous findings that cholesterol is required for synaptic connections. We further show that Ptc does not function directly in this process but via smoothed, a positive effector of Hedgehog-signaling. These results reveal a role for Ptc in preventing neurodegenerative disease in adult *Drosophila* and raise the possibility of aberrant synaptic transmission in the development of such brain diseases.

D2-002

Protein modifications affect Huntington's disease pathogenesis in *Drosophila*

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Several late-onset human neurodegenerative diseases including Huntington disease are caused by expanded repeats of CAG that encode polyglutamines within the disease protein. No treatment for these agonizing and lethal diseases exists, a problem made more difficult by not knowing the pathogenic target of the expanded glutamine repeat. Here we will describe our recent and ongoing studies using a *Drosophila* model system that faithfully mimics the human disease to explore mechanisms of pathology and development of therapeutics. We find that several acetyltransferases are targets that are bound and/or inhibited by mutant Huntingtin (Htt) and we will address the specificity of HATs and HDACs in the pathogenic process of HD. Experiments leading to the development of improved therapeutics will be described. Experiments describing the modification of the pathogenic fragment of Huntingtin (Httex1p) by either SUMO-1 or ubiquitin will be presented. We find in a *Drosophila* model of HD, that SUMOylation exacerbates while ubiquitination relieves neurodegeneration. Mutations that prevent both SUMOylation and ubiquitination dramatically reduce pathology, indicating that the contribution of SUMOylation extends beyond simply preventing ubiquitination. We will discuss continuing experiments that address the molecular mechanisms of this modification and illuminate potential therapeutic targets. We will also describe experiments that identify novel combinatorial strategies of potential therapy.

D2-003

Genomic analysis of JNK signaling and dorsal closure in *Drosophila*

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The *Drosophila* Jun N-terminal kinase (JNK) signaling pathway controls several important processes during development including epithelial morphogenesis, wound-healing, planar cell polarity, apoptosis, and the immune and stress responses. Dorsal closure (DC) of the embryo is a paradigm to study how JNK signaling controls epithelial morphogenetic movements. Dorsal closure takes place during mid-embryogenesis to cover the dorsal surface

of the embryo with a monolayered epithelium which ultimately will give rise to the dorsal epidermis. This tissue movement shares several similarities with wound-healing. Despite a good knowledge of the cytoplasmic signaling events leading to Jun/Fos (AP-1) activation, only a very limited set of target genes is known to control biological processes downstream of JNK activation. We have used Affymetrix DNA microarrays to identify genes that are regulated by JNK signaling during embryonic dorsal closure. Comparison of the transcriptome from wild type vs. JNKK/Hemipterous-activated embryos led to the identification of a set of approx. 500 genes which are either positively or negatively regulated by a fold change of at least 2. Ongoing experiments using this large set of data, together with the functional, including quantitative, analysis of selected novel JNK target genes, will be presented.

D2-004

Histone methylation and the control of gene silencing in *Drosophila*

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Genetic dissection of gene silencing in position-effect variegation (PEV) in *Drosophila* by Su(var) and E(var) mutations allowed identification and molecular characterization of genes controlling epigenetic processes. Several of these genes encode histone methyltransferases (HMTases) responsible for indexing chromosomal subdomains by lysine methylation in histone H3 and H4. In *Drosophila*, H3-K9 di- and trimethylation is mainly controlled by the SU(VAR)3-9 HMTase, whereas all three H3-K27 methylation states are independently mediated by E(Z). Trimethylation of H4-K20 in heterochromatin depends on H3-K9 methylation by SU(VAR)3-9 and is catalysed by the SUV4-20 HMTase. Mutational analysis of SU(VAR)3-9 demonstrates that its silencing potential in heterochromatin depends on its HMTase activity. Notably, a hypermorphic Su(var)3-9 mutant displays extensive H3-K9 di- and trimethylation also outside constitutive heterochromatin and causes ectopic heterochromatinization and gene silencing. SU(VAR)3-9 forms complexes with the heterochromatin protein HP1. By interaction with other HMTases the SU(VAR)3-9/HP1 complex directs other histone methylation marks to heterochromatin. Analysis in fission yeast, *Drosophila*, mammals and plants demonstrate that SU(VAR)3-9 dependent gene silencing processes are conserved throughout evolution. Consequently, *Drosophila* Su(var)3-9 mutations are rescued by human SUV39H1 transgenes. Heterochromatinization of chromosomal domains depends on removal of histone modifications indexing euchromatin. Su(var)3-1 mutations impair Su(var)3-9 mediated heterochromatic gene silencing. Su(var)3-1 was identified as antimorphic mutants of the H3-S10 kinase JIL-1. Although JIL-1Su(var)3-1 mutants maintain kinase activity they strongly impair expansion of heterochromatic structures into euchromatin. These mutants indicate a general effect of JIL-1 in controlling heterochromatin expansion and provide evidence for dynamic regulation of the balance between heterochromatin and euchromatin.

D2-005

Signalling cross-talk during vulval development in *Caenorhabditis elegans*

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The proper coordination of signals from different genetic pathways is crucial for cell fate specification during animal development. The

vulva of the nematode *Caenorhabditis elegans* is an important paradigm for cell-cell interactions and cell fate determination. The *C. elegans* vulva develops from a subset of six ectodermal vulval precursor cells (VPCs, consecutively numbered P3.p-P8.p). An inductive signal from the anchor cell of the gonad activates a canonical RTK/Ras/MAPK signalling cascade in the closest VPC, P6.p, to promote the primary vulval fate. A lateral signal then activates a LIN-12/Notch receptor-mediated pathway in the adjacent cells P5.p and P7.p to prevent them from adopting the primary fate and instead to specify the secondary vulval fate. Understanding how the inductive and lateral signalling pathways are coordinated is the key to understand how VPC fates are specified, forming a precise spatial pattern of the vulval tissue. I show that reduced activity of genes encoding the HOX protein LIN-39 and its PBX/EXD-like co-factor CEH-20 results in compromised LIN-12/Notch-mediated lateral signalling. Inactivation of either *lin-39* or *ceh-20* suppresses the multivulva phenotype of *lin-12(n137)* gain-of-function mutant animals. Furthermore, the LIN-39/CEH-20 complex is required for the expression of both LIN-12 and its ligand LAG-2 in the VPCs prior to and during vulval induction, and LIN-39 binds to the *lin-12* and *lag-2* promoters *in vivo*. These data imply that LIN-39/CEH-20, which functions at the bottom of the RTK/Ras/MAPK, Wnt and SynMuv signalling cascades during vulval induction, serves as a major integration site and relay in transmitting signals to the LIN-12/Notch pathway to specify vulval cell fates.

D2-006

How brassinosteroid-biosynthetic enzymes control plant morphogenesis?

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Brassinosteroids (BRs) are steroidal plant hormones that control important processes of growth and development. BR-deficient mutants show dwarfed stature, impaired photomorphogenesis, weak germination and male sterility. The level and homeostasis of the biologically active hormone is controlled by feedback regulation acting negatively on biosynthesis, the main determinant of BR accumulation, and positively on the degradative processes. In Arabidopsis constitutive photomorphogenesis and dwarfism (CPD) is a cytochrome P450-type monooxygenase that catalyzes the rate-limiting C-23 side-chain hydroxylation reaction of BR synthesis. Earlier we found that the expression of CPD and several other BR-biosynthetic enzymes, all belonging to the CYP90 and CYP85 P450 families, are regulated primarily at the level of transcription. Whereas all CYP90 and CYP85 genes are highly active during germination, each of them shows a characteristic developmental and organ-specific expression pattern. Because active BRs are not transported within the plant, localized expression of these enzymes can determine the sites of hormone synthesis and, as a result, influence tissue and organ differentiation. We followed the activities of BR-biosynthetic genes and the accumulation of their transcripts using reporter gene constructs and RT-PCR. CPD and CYP85A2, the enzyme producing bioactive BRs, were found preferentially expressed in the apical meristeme and differentiating leaves. CYP85A2 was also active during fruit development, and its transcript level closely correlated with the accumulation of the active hormone. The specific patterns of CYP90 and CYP85 gene activities are in good agreement with the observation that, although a lesion in each of these genes results in BR-deficiency, each mutant has unique phenotypic traits.

D2-007P

Molecular biological characterization and functional analysis of PP13/galectin-13

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Placental protein 13 (PP13) was cloned from human term placental cDNA library. As sequence analyses, alignments and computational modelling showed its homology to members of the galectin family, the protein was designated galectin-13. The protein was found to be a homodimer of 16 kDa subunits. Phosphorylation sites were computed and phosphorylation of the purified protein was confirmed. Similarly to human eosinophil Charcot Leyden Crystal protein/galectin-10, its weak endogenous lysophospholipase activity was proved by 31P NMR. Sugar binding assays revealed that N-acetyl-lactosamine, mannose and N-acetyl-glucosamine residues widely expressed in human placenta had the strongest binding affinity to PP13/galectin-13, which also effectively agglutinated erythrocytes. Using affinity chromatography, PAGE, MALDI-TOF MS and PSD, annexin II and beta/gamma actin were identified as proteins specifically bound to PP13/galectin-13. Perinuclear staining of the syncytiotrophoblasts showed its expression in these cells, while strong labeling of the syncytiotrophoblasts' brush border membrane confirmed its galectin-like externalization. The encoding sequence of PP13/galectin-13 was inserted into pcDNA3 vector and U937 cells were transfected. As it was found by MTT-test, cells overexpressing PP13/galectin-13 were extremely sensitive to various stress effects, such as low concentration of hydrogen-peroxide or taxol compared to control cells. Western-blot results showed a remarkable activation of MAPK-pathways and pro-apoptotic markers in case of PP13/galectin-13 overexpression. Cells were also transfected with a PP13-GFP fusion plasmid to confirm the localization of the protein previously seen by immunohistochemistry. With regard to our functional and immunomorphological results, PP13/galectin-13 may have special immunobiological function at the lining of the common feto-maternal blood-spaces and developmental role in the placenta by promoting or mediating apoptotic events.

D2-008P

Negative transcriptional modulation and silencing of the bi-exonic Rnf35 gene in the preimplantation embryo by the CCAAT-displacement protein (CDP)/Cux

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The mouse Rnf35 gene is bi-exonic in structure and is transcribed using an initiator core promoter in the preimplantation embryo until it is permanently silenced between the eight-cell and the blastocyst stages of development. We have previously shown that Rnf35 transcription is positively regulated by the nuclear factor Y.

Using the uniquely permissive CHO-K1 cell line in transient transfection assays, and in micro-injection of mouse embryos, we demonstrate in this work that the Rnf35 promoter is negatively modulated by a *cis*-cognate repressor element, designated as the downstream exon 1 repressor, or DER, residing between +72 and +95 in the untranslated exon 1 of the Rnf35 gene. Simultaneous mutagenesis of the two half-sections, DER1 and DER2, of the DER sequence resulted in derepression suggesting participation of multiple proteins in the observed DER-dependent transcriptional repression. Electrophoretic mobility shift assays further reveal that the 3'-half of DER (DER2) is targeted by the repressor CCAAT-displacement protein (CDP)/Cux; the DER-dependent repression is partially relieved *in vivo* in co-transfection with an antisense CDP construct. Expression profile analysis further shows that transcription of the Cdp gene first occurs between the eight-cell and the blastocyst stages in the mouse, correlating and possibly explaining the onset of Rnf35 silencing. Taken together, our results suggest that the evolutionarily acquired untranslated exon 1 of Rnf35, and possibly those of other similarly structured bi-exonic early embryonic genes, contributes to transcriptional modulation in the preimplantation embryo.

D2-009P

Site-directed mutagenesis of putative metal ligands of manganese lipoxygenase

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Manganese lipoxygenase (MnLOX) belongs to the lipoxygenase gene family and it is the only known lipoxygenase with catalytic Mn instead of Fe. MnLOX transforms linolenic acid to 11S-hydroperoxylinolenic (11S-HPOTrE) and to 13R-hydroperoxylinolenic (13R-HPOTrE) acids, and thus belongs to the group of R-lipoxygenases. MnLOX is secreted by the take-all fungus of wheat, *Gaeumannomyces graminis*. We expressed Mn-LOX in *Pichia pastoris* and site-directed mutagenesis of putative metal ligands was performed by PCR technology using HPLC-purified primers and Pfu-1 polymerase (Quick Change protocol). *Pichia pastoris* in fermentor secreted ~30 mg Mn-LOX L-1 culture medium. The recombinant MnLOX contained ~1 mole Mn per mole protein. It was N- and O-glycosylated and had similar kinetic properties as the native MnLOX. The iron ligands of soybean lipoxygenase-1 are three His residues, a distant Asn residue, and the C-terminal Ile residue. The His274Gln, His278Glu, His462Glu, and the Val-602 deletion mutants of MnLOX were inactive, and had lost >95% of the Mn content. His-463, Asn-466 and Gln-467 did not appear to be critical for MnLOX activity, as His463Gln, Asn466Gln, Asn466Leu, and Gln467Asn mutants metabolized α -linolenic acid to 11- and 13-HPOTrE. Many lipoxygenases, which form hydroperoxides of S-configuration, have a conserved Ala residue, whereas R-lipoxygenases seems to have a conserved Gly residue in the same position. We studied whether mutation of the corresponding residue, Gly-316, to Ala of MnLOX might change its catalytic properties. The mutant Gly316Ala metabolized α -linolenic acid to 13R-HPOTrE as the main product with formation of only small amounts of 9S-HPOTrE. Gly316A rapidly transformed 13R-HPOTrE to 13-ketolinolenic acid and to epoxy alcohols, under normal oxygen atmosphere (0.22 mM O₂).

Conclusions: His-274, His-278, His-462, and Val-602 likely coordinate Mn in agreement with the alignment predictions of MnLOX with the metal ligands of iron lipoxygenases. The Gly316Ala mutant of MnLOX likely changed the position of 13R-HPOTrE in the active site, leading to the homolytic cleavage of the peroxide by the metal and formation of epoxy alcohols and 13-ketolinolenic acid.

D2-010P

A novel, evolutionarily conserved role in intracellular signalling for the gelsolin-related actin-binding protein Flightless I

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The *flightless I* (*FliI*) gene encodes a conserved gelsolin-related actin-binding protein (GRABP) essential for early embryogenesis in mammal and fly. The mammalian FliI protein is a coactivator of nuclear hormone receptor (NR)-mediated transcription. FliI interacts synergistically with NRs and NR coactivators including the type I protein-arginine methyltransferase PRMT4/CARM1 which modifies histone structure at the promoters of target genes through methylation. Yeast two-hybrid analysis showed that mammalian and *C. elegans* homologues of FliI also interact with the respective FLAP (FliI LRR-associated protein) homologues. *FliI* and *FLAP* exhibit similar RNAi phenotypes in *C. elegans* involving abnormal gonad morphology and function, and lowered viability, supporting their role in a common pathway of action. In *C. elegans* and mammals, type I protein-arginine methyltransferases were found to associate with FLAP in addition to FliI. Microarray studies on *FliI* knockdown worms revealed that many genes upregulated in response to suppression of *FliI* encode proteins likely to function in the nucleus. As well as actin, FliI also binds actin-related protein 4 (Arp4/BAF53) *in vivo*. BAF53 is a nuclear protein found in SWI-SNF-related chromatin remodelling complexes. BAF53 homologues exhibit conservation of residues that, in actin, bind to GRABPs, indicating that the BAF53 interaction with FliI is evolutionarily conserved. Recent studies indicate that other GRABPs including supervillin and gelsolin are involved in NR-mediated transcriptional control. We are examining the role of GRABPs in aspects of mammalian wound healing using *FliI* knockout and transgenic mice; our studies indicate that FliI is an important mediator of wound repair.

D2-011P

The PI3K/Akt pathway regulated by IGF-I and PTEN is essential for differentiation of neural stem cells

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The control of neurogenesis in the central nervous system (CNS) involves regulation of proliferation, migration, cell death and differentiation of neural stem cells. In mice, the population of locally born stem cells from the embryonic olfactory bulb (OBSC) depends on (pro)insulin or insulin-like growth factor-I (IGF-I)

for survival and proliferation in culture. *In vivo* and in culture, the OBSC require IGF-I for differentiation to neurons, astrocytes and oligodendrocytes. We analysed the implication of the PI3 kinase pathway, its substrate Akt and the phosphatase PTEN, a negative regulator of this signalling pathway, in the proliferation and differentiation of OBSC. Upon stimulation with 100 ng/ml of IGF-I for 30 min, there is a marked increase in P-Akt^{ser473} and P-Akt^{thr308} in proliferating OBSC as well as in cells undergoing differentiation for 48 h. In contrast, there is no further activation of ERK1/ERK2, markedly phosphorylated in the basal state. IGF-I knockout E16.5 and E18.5 embryos have lower levels of P-Akt^{ser473} in the olfactory bulb *in vivo*. Overexpression of PTEN, using a retroviral construct with a Green Fluorescence Protein tag during the proliferative phase of OBSC, did not change the proportion of cells which incorporated BrdU, whereas the number of neurons and astrocytes was decreased, when the culture was maintained in the absence of insulin. The PTEN overexpression induced in parallel a decrease of the basal levels of P-Akt^{ser473} and P-Akt^{thr308} without changing total Akt levels. These data support that IGF-I activates the PI3 kinase/Akt pathway during neurogenesis in the olfactory bulb *in vivo* and *in vitro*. The phosphatase PTEN appears as a balancing molecule for neural differentiation of OBSC.

D2-012P

Characterization, expression and functional aspects of human ninein isoforms

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The centrosome plays key roles in the formation of the mitotic spindle, cell polarity and cell locomotion. The centrosomal-associated protein, hNinein, has been identified as a microtubules minus end capping, centrioles position, centrosome maturation and anchoring protein. In this report, we examine whether four C-terminal of hNinein splicing isoforms, including isoform 1, isoform 2, isoform 5 and a newly finding isoform 6, represent differential characterization, expression and functional aspects. To explore the possible biological functions of hNinein isoforms, their distribution and expression levels were analyzed in adult multiple tissues. Isoform 1, 2 and 5 could be amplified as expect in adult multiple tissues using RT-PCR. Besides, isoform 6 could be amplified in brain, skeletal muscle and heart. We speculate that isoform 6 might play a role and exist in post-mitotic cell including muscle or nerve cell. Using immunofluorescence assay, we show that hNinein isoform 1, 2 and 5 are concentrated at centrosomes in HeLa cells, whereas isoform 6 is distributed to the nuclear membrane. Overexpression of all four hNinein isoforms in HeLa cell might influence the distribution of gamma-tubulin. *In vitro* kinase assay show that differential phosphorylation capacity of hNinein isoforms by GSK3beta but not Aurora A and PKA. In summary, we found C-terminal of four hNinein isoforms, having differential in tissue distribution, cell localization and phosphorylation capacity. These isoforms might play important roles in physiological function and cell development.

D2-013P

Determination of the neurotransmitter phenotype during the *in vitro* differentiation of NE-4C neuroectodermal stem cells

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The regional localization of a neuron is known to correlate with the establishment of its neurotransmitter phenotype, but the underlying mechanisms are far from clear. Possible interactions of position-regulating genes with genes determine the neurotransmitter phenotypes were investigated using the *in vitro* induced differentiation of cloned NE-4C neuroectodermal stem cells. Non-differentiated NE-4C cells express anterior neuroectodermal markers Sox-2, Otx-2 and En-1. Otx2 and En1, however, were expressed also by embryonic stem (ES) cells and indicated a rather general early anterior stem cell fate. Several region-specific genes were not active in non-induced cells, but got activated in defined phases of neuronal differentiation. We found significant increase in the expression of a dorsal (Emx2) and a ventral (Dlx2) fore-brain marker, in the activation of the midbrain-specific Otx3, the midbrain-hindbrain marker Gata-2/3 and SCL and the hindbrain-specific Gbx2 and Hoxb2. In stem cells re-cloned from differentiated NE-4C cultures, Emx2 or Hoxb2 were not expressed, but – as in the mother clone – they got activated in the course of neural differentiation. The activation of several region specific genes indicated that NE-4C stem cells were not positionally determined. Differentiated NE-4C neurons displayed both GABAergic and glutamatergic phenotypes. Key genes of the glutamatergic (Vglut2) and the GABAergic phenotype (GADs), however, were detected only in cultures containing mature neurons and expressing also Emx2 and Dlx1. The data suggest that the glutamatergic and GABAergic phenotype is determined in parallel with the regional commitment. As a consequence, non-committed neuroectodermal stem cells may develop multiple transmitter phenotypes.

D2-014P

The expression of hemoglobin genes in development of hematopoietic cells differentiated from human embryonic stem cells

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Embryonic stem cells are interesting cells in study of development. They are self-renewing and pluripotent cells which can differentiate to all three germ layers. Thus, study of embryonic stem cell differentiation can result in the understanding of processes that take place in embryonic and fetal stages of hematopoiesis. For these reasons, we differentiated the human embryonic stem cells, Royan H1, to hematopoietic lineage in feeder free system. The presence of feeder, which is usually mouse embryonic fibroblast, can conduct development of human embryonic stem cells to an inappropriate direction. In the different days after differentiation, cells were harvested and RT-PCR accomplished. The results showed that in the early days differentiated cells only express ζ and ϵ hemoglobin genes whereas in day 14, α , γ and δ genes were expressed in addition to ζ and ϵ because the population of differentiated cells was heterogeneous. So, *in vitro* development showed a switching from embryonic hemoglobin genes to fetal ones. In conclusion, embryonic stem cells can be considered as invaluable *in vitro* model in developmental studies of hematopoietic system.

D2-015P**The role of nucleophosmin/B23 in the development of thymus: characterization of B23 by proteomic tool**T.-L. Pan¹, P.-W. Wang¹, H.-W. Chiu¹ and B. Y. Yung¹¹*Proteome & Chinese Medicine Laboratory, School of Traditional Chinese Medicine, Chang Gung University, Tao-Yuan, Taiwan ROC*, ²*Cancer Biochemistry Laboratory, Department of Pharmacology, Chang Gung University, Tao-Yuan, Taiwan ROC*.
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Nucleophosmin/B23 is one of the major nucleolar phosphoproteins which plays a critical role in increased nucleolar activity that is necessary for cell proliferation. Nucleophosmin/B23 has also been ascribed a number of diverse properties, including cytoplasmic/nuclear shuttle protein applied as a substrate of proteins kinase C and its potential roles in RA-induced differentiation and apoptosis of cells have been concerned recently. However, the functional association of nucleophosmin/B23 with cellular apoptosis/differentiation is still controversial. The thymus is an encapsulated gland that functions as a primary lymphoid to generate T cells repertoire and proceed to select specific types of mature T cells from immature thymocyte precursor. The molecular processes have been proven to undergo cellular proliferation, differentiation and apoptosis in series which might be involved in regulation of transcription factors (Rel/NF- κ B) in thymus. We speculate these transcription factors binding to nucleophosmin/B23 would associate with the genes activated for above cellular modifications. In this study, we used thymus as a model to characterize the mechanisms of nucleophosmin/B23 participating in T-cell development by two-dimensional electrophoresis (2-DE) and 2-DE immunoblotting analysis. The recognized proteins were identified by peptide mass fingerprint resolution after matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry and de-novo sequencing followed LID dissection. The expression and localization of these proteins in the thymus cells was evaluated with IHC staining. Based on the results, we exploited PCNA would induce cell differentiation/apoptosis through interaction with B23 molecule in thymus.

D2-016P**Functional analysis of seven genes encoding eight translation initiation factor 4E (eIF4E) isoforms in *Drosophila***G. Hernández¹, M. Altmann², J. M. Sierra³, H. Urlaub⁴, R. Diez del Corral³, P. Schwartz⁵ and R. Rivera-Pomar¹¹*Abt. Molekulare Biologie, Max-Planck Institut für Biophysikalische Chemie, Göttingen, Germany*, ²*Institut für Biochemie und Molekularbiologie, Universität Bern, Bern, Switzerland*, ³*Centro de Biología Molecular Severo Ochoa, Universidad Autónoma de Madrid, Madrid, Spain*, ⁴*Abt. Zelluläre Biochemie, Max-Planck Institut für Biophysikalische Chemie, Göttingen, Germany*, ⁵*Abt. Anatomie-Embryologie, Zentrum Anatomie, Georg-August Universität Göttingen, Göttingen, Germany*.
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The *Drosophila* genome-sequencing project has revealed a total of seven genes encoding eight eukaryotic initiation factor 4E (eIF4E) isoforms. Four of them (eIF4E-1,2, eIF4E-3, eIF4E-4 and eIF4E-5) share exon/intron structure in their carboxy-terminal part and form a cluster in the genome. All eIF4E isoforms bind to the cap (m⁷GpppN) structure. All of them, except eIF4E-6 and eIF4E-8 were able to interact with *Drosophila* eIF4G or eIF4E-binding protein (4E-BP). eIF4E-1, eIF4E-2, eIF4E-3, eIF4E-4 and eIF4E-7 rescued a yeast

eIF4E-deficient mutant *in vivo*. Only eIF4E-1 mRNAs and, at a significantly lower level, eIF4E-3 and eIF4E-8 are expressed in embryos and throughout the life cycle of the fly. The transcripts of the remaining isoforms were detected from the second instar larvae onwards. This indicates the cap-binding activity relies mostly on eIF4E-1 during embryogenesis. This agrees with the proteomic analysis of the eIF4F complex purified from embryos and with the rescue of I(3)67Af, an embryonic lethal mutant for the eIF4E-1,2 gene, by transgenic expression of eIF4E-1. Overexpression of eIF4E-1 in wild-type embryos and eye imaginal discs results in phenotypic defects in a dose-dependent manner.

D2-017P**Aldehyde dehydrogenase activity in C2C12 myogenic cell line**

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The aldehyde dehydrogenase enzyme is expressed highly in hematopoietic stem and progenitor cells. This enzyme plays a significant role in retinoic acid metabolism known to be involved in diverse biological processes such as embryogenesis, growth, and differentiation. Aldehyde dehydrogenase activity can be used successfully to enrich for mouse and human hematopoietic cells with primitive progenitor cell surface phenotype. We investigate the aldehyde dehydrogenase activity in the C2C12 cell line to assess whether this activity could be used for the isolation of genuine myogenic stem cells. Indeed, their phenotype is obscure and their isolation remains elusive. We show that a subpopulation of cells with high aldehyde dehydrogenase activity (ALDhi) could be found in 0.5–2% of C2C12 total cells. Furthermore, treatment with histone deacetylases inhibitors leads to the increase of this ALDhi C2C12 cell subpopulation. Interestingly, sorted C2C12 cells with low aldehyde dehydrogenase activity (ALDlo) generate rapidly in culture ALDhi cells. This suggests a dynamic equilibrium among cells with different enzymatic activity. Results on the *in vitro* and *in vivo* differentiation capacity of the different subpopulations will be presented.

D2-018P**Post-transcriptional regulation of CCN2/CTGF gene expression during differentiation of chicken chondrocytes: involvement of a putative trans-factor which interacts with a cis-element in the 3'-UTR of mRNA**Y. Mukudai^{1,2}, S. Kubota¹, T. Eguchi¹, S. Kondo¹, K. Nakao¹ and M. Takigawa^{1,2}¹*Biochemistry and Molecular Dentistry, Okayama University Graduate School of Medicine and Dentistry, Okayama, Okayama Japan*, ²*Biodental Research Center, Okayama University Dental School, Okayama, Okayama Japan*.
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We have revealed that a multifunctional growth factor CCN2/CTGF is highly expressed in prehypertrophic-hypertrophic chondrocytes and plays important roles in growth and differentiation of chondrocytes, and that the 3'-untranslated region (3'-UTR) of CCN2 mRNA contains a cis-repressive element of gene expression. In the present study, we found that gene expression of CCN2 is regulated at post-transcriptional level depending

on differentiation stages of chicken chondrocytes. Reporter gene assay revealed that the minimal repressive cis-element of the 3'-UTR of chicken CCN2 mRNA was located within the area between 100 and 50 bases from the polyadenylation tail. Moreover, the stability of CCN2 mRNA was correlated with the interaction between the cis-element and a 40-kDa-trans-factor in nuclei and cytoplasm. In fact, the binding was prominent in proliferating chondrocytes, and attenuated in (pre)hypertrophic chondrocytes. Stimulation by bone morphogenetic protein-2, platelet-derived growth factor and CCN2 stabilized CCN2 mRNA in proliferating chondrocytes, whereas it destabilized the mRNA in prehypertrophic-hypertrophic chondrocytes. Interestingly, stimulation by the growth factors repressed the binding in proliferating chondrocytes, however, it enhanced it in (pre)hypertrophic chondrocytes. Therefore, gene expression of CCN2 mRNA during chondrocyte differentiation toward endochondral ossification is properly regulated, at least in part, by the changing stability of the mRNA, which arises from the interaction between the RNA cis-element and putative trans-factor. We will also report on purification and characterization of the putative trans-factor.

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D2-019P

The developmental proteome of the mouse retina

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The developing vertebrate retina is a well characterized structure that has been widely used as a model to study nervous system development. The retina contains five basic cell types that originate from a common retinal progenitor cell. These five cell types are generated in a characteristic yet overlapping order that is conserved across vertebrate species. The adoption of cell fate is dependent upon both expression of appropriate receptors and transcription factors by the progenitors as well as expression of appropriate secreted factors, cell-surface molecules and extracellular matrix molecules by earlier born retinal neurons. In order to begin to better understand the coordination of such complex developmental events, we have characterized the proteome of the developing mouse retina. Protein samples were taken from developing mouse retinas aged embryonic day 13 (E13), E15, E17, E18, postnatal day 1 (P1; day of birth), and P5 and separated by two-dimensional gel electrophoresis. Gels were stained with a protein specific stain, imaged, and the relative abundance of each protein spot was analyzed across developmental time. Analysis using self-organizing mapping and adaptive resonance theory was used to cluster protein spots into groups based on dynamic changes in their levels of expression. In addition to clusters of proteins that generally increased or decreased, several clusters had peak expression levels that temporally correlated with the peak genesis of specific retinal cell types. These results represent the first efforts to use proteomics to investigate retinal development, and are the basis for future biological and computational efforts to understand the coordination of multiple cell-specific events required for proper function of this vital organ.

D2-020P

Application of immunoenzymology in detection of bone morphogenetic proteins

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Bone morphogenetic proteins (BMPs) belong to TGF- β superfamily of growth factors. Most of BMPs have demonstrated the ability to induce new bone formation. This evoked the interest in their application in bone reconstruction and healing processes. Purification of these proteins, especially in large quantities is difficult. One of the reasons is lack of a reliable method, which enables the monitoring of the purification procedure. In most cases *in vivo* assays, especially rat subcutaneous assay, are used. This prolongs purification procedure because the results are known only after several weeks. Our studies concentrated on the preparation of an immunoenzymatic method that would be able to detect small quantities of BMP in diluted fractions obtained during purification. We purified bone morphogenetic protein using modification of Luyten method. Samples from each step of purification (hydroxyapatite chromatography, Sephacryl S-200) were tested for their osteoinductive potential using rat subcutaneous assay. The same fractions were also subjected to devised by us immunoenzymatic method involving primary mouse anti-bovine BMP IgG and secondary anti-mouse IgG from goat, conjugated with horse radish peroxidase. The created by us method was efficient in precise and fast localization of the BMP in the same fractions that displayed osteoinductive properties and did not give false positive results.

D2-021P

Proteome analysis of anther-development-related proteins in a thermo-sensitive male sterile rice mutant

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In this study, the differences in protein profiles between the anthers of a genic thermo-sensitive male sterile rice mutant (AN S-1) and its wild type (AN-N) were displayed in classical two-dimensional (2DE) electrophoresis gels; and tryptic fragments fingerprinting in MALDI-TOF mass spectroscopy (MS) was employed to identify the proteins that showed differential expression. Since the comparison was made at the late stage of anther development, the differences may reveal those differentially expressed proteins (mostly down-regulated in the mutant) which possibly play a role in normal anther development. A detailed comparison of protein patterns between AN-N and AN S-1 grown at high temperature was made. It was found that 446 protein spots were regarded as significantly changes in expression level (> 2 folds) between AN-N and AN S-1. Among which 60 protein spots were investigated and 31 protein spots were identified successfully. The down-regulated proteins found in AN S-1 include those involved in hydrogen peroxide detoxification, protein folding maintenance, ATP synthesis, carbohydrate metabolism, cytoskeleton formation and starch synthesis. These results seem to suggest that the accumulation of cytosolic hydrogen peroxide might impair ATP synthesis in anthers of AN S-1 at high temperatures; the subsequent low energy status thereby cannot fulfil the energy demand for anther development and eventually will lead to pollen abortion. This study points to the possibility of hydrogen peroxide involvement in male sterility in AN S-1 mutants.

D3 – Proteins Linking Innate and Adaptive Immunity

D3-001

Carbohydrate-binding receptors in innate immunity

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Sugar-binding receptors on cells of the immune system mediate innate immunity. Biochemical, structural and genomic analysis of this family of receptors suggests that each has a distinct role in pathogen recognition. Two of the best understood members of the family serve as models for how sugar binding can lead to biologically significant interactions with the immune system. Serum mannose-binding protein (MBP) initiates the lectin branch of the complement pathway when it interacts with surfaces of fungi or bacteria and activates MBP-associated serine proteases (MASPs). The MASPs bind to a collagenous stalk region of MBP. The significance of this pathway is indicated by susceptibility to infections observed in individuals with variant forms of MBP in which the collagenous region is destabilized. Activation of the proteases involves subtle conformational changes in the collagen stalks when the sugar-binding domains attach to a pathogen. MBP distinguishes pathogens from the host by binding to mannose and N-acetylglucosamine, which are commonly found on bacterial and fungal surfaces but are much rarer on mammalian surfaces. Further discrimination is achieved by the requirement for multivalent binding in a fixed geometrical arrangement such as that found on surfaces. The dendritic cell receptor DC-SIGN and the closely related endothelial cell receptor DC-SIGNR (L-SIGN) bind glycans containing mannose in a different way. Extended binding sites generate relatively high affinity for oligosaccharide ligands of the type found on viral surface glycoproteins. Flexibility in the orientation of CRDs allows multiple interactions with glycans on a single target glycoprotein. The ability to bind but release viruses accounts for the role of these receptors in presenting human immunodeficiency virus to T cells. Thus, subtle differences in the structures of receptors containing C-type carbohydrate-recognition domains can lead to binding of very different classes of glycan-bearing pathogens.

D3-002

Toll-like receptor signal transduction

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Toll-like receptors have emerged as key initiators of host defence against pathogens. They recognize a large number of microbial products, providing a repertoire that allows the host to respond to all invading pathogens. Once they are activated they trigger signalling pathways that culminate in the increased expression of a large number of immune and inflammatory genes. The best characterized TLRs are TLR4, which recognizes LPS, and TLR3 which responds to viral double-stranded RNA. Signalling is driven by the toll-IL-1 receptor (TIR) domain, which occurs in all TLRs and also in the four adapter proteins implicated in receptor-proximal signalling MyD88, Mal, Trif and Tram. These adapters are probably recruited to receptor TIR domains, which form a dimeric platform allowing assembly of the initial signalling complex. The adapters then recruit down-stream effectors, which include three families of kinases: IRAKs, RIPs and TBK-1. Signalling specificity is becoming apparent with TLR3 recruiting Trif, which in turn activates RIP1 and TBK-1, leading

to activation of IRF-3. Most other TLRs recruit MyD88, which activates IRAK-4 and RIP2, leading to activation of NF- κ B induction of multiple inflammatory genes. This allows for tailoring of the response to invading pathogens and provides specificity to innate immunity, a feature that is becoming increasingly apparent. Specific roles for Mal and Tram have yet to emerge although there are clear biochemical differences between these and the other adapters. Tram is myristoylated and recently we have found that Mal is a substrate for caspase-1 and required cleavage in order to be activated. Endogenous inhibitors of TLRs have also been described which function by sequestering adapters from the signalling pathways, a notable example being ST2. Clearly we have much to learn about the component parts in TLR signalling and their regulation.

D3-003

Killer-cell immunoglobulin-like receptors

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Killer-cell immunoglobulin-like receptors (KIR) are a family of immune-system receptors expressed on the surface of natural killer (NK) cells and subpopulations of T cells. KIR thus function in both the innate immune response and the adaptive immune response. The known ligands for KIR are major histocompatibility complex (MHC) class I molecules. Best characterized are inhibitory receptors that recognize two types of HLA-C ligand, specificities that are largely determined by single amino-acid substitutions in the receptor and the ligand. The inhibitory HLA-C specific KIR are major components of the mechanism that ensures NK cells are tolerant of healthy cells, but responsive to cells that are virally infected or stressed in other ways. They also appear to contribute to reproduction, by influencing the remodeling of maternal blood vessels that is necessary to feed the fetus. Despite these functions HLA-C and its cognate KIR are of recent evolution, being present only in humans and some apes. This exemplifies the rapid evolution and species-specificity of the KIR system. Particularly short-lived are the activating KIR, for which ligands and functions are poorly understood, although correlative clinical studies point to their contribution to the immune response and autoimmunity.

D3-004

The β subunit of the type I Fc ϵ receptor is a target for complement-derived peptides inhibiting IgE-mediated secretory response of mast cells

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Peptides originally derived from complement C3a earlier were shown to inhibit the type I Fc ϵ receptor (Fc ϵ RI)-mediated degranulation of mucosal type mast cells. Here we show that C3a7, a peptide with a natural sequence, and its modified derivative

C3a9, are powerful inhibitors of the IgE-mediated response in both serosal and mucosal type mastocytes. We demonstrate that these peptides inhibit FcεRI-clustering induced membrane-proximal events: suppress phosphorylation of the FcεRI β subunit, the protein tyrosine kinase Lyn, as well as the transient rise in free cytosolic Ca²⁺-level. The late phase of cellular response was also inhibited, as demonstrated by the reduced tumor necrosis factor α (TNF-α) secretion. Evidence from two independent methods show that the interaction site of complement-derived peptides is the β-chain of FcεRI. This was further supported by fluorescence confocal microscopic colocalization data. Based on the presented data we propose separate “activating” and “inhibitory” sequences in C3a, which are in balance under physiologic conditions. Results shown suggest that derivatives of the inhibitory peptides are potent agents for future therapeutic interventions.

D3-005

Expression of Fc gamma RIIA in PLB cells during differentiation by 1,25(OH)₂D₃ depends on cytosolic PLA₂ and is regulated via activation of CREB by PGE₂

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The requirement of cytosolic phospholipase A₂ (cPLA₂) for Fc gamma RIIA expression was studied in a model of deficient PLB-985 cells (PLB-D). Fc gamma RIIA was expressed in differentiated parent PLB cells by 1,25 dihydroxyvitamin D₃ (1,25(OH)₂D₃), retinoic acid and interferon gamma, but not in differentiated PLB-D cells. In contrast, Fc gamma RI, Fc gamma RIII and complement receptors were similarly expressed in parent differentiated PLB cells and PLB-D cells. While addition of exogenous PGE₂ (12 μM) to PLB-D cells during differentiation with 1,25(OH)₂D₃ caused a significant restoration of Fc gamma RIIA expression, addition of indomethacin (30 μM) inhibited the release of PGE₂ and the expression of Fc gamma RIIA. The expression of EP₄, the inhibition of Fc gamma RIIA expression by the PKA inhibitor, H-89 (10 μM) during differentiation of PLB cells and induction of FcγRIIA expression by dbcAMP in PLB and PLB-D cells, suggest the activation of the PKA pathway in Fc gamma RIIA induction. CREB phosphorylation and CREB-CRE interaction were detected at 2 days of differentiation of parent PLB cells which coincided with the kinetics of PGE₂ release and EP₄ development, but were not detected in PLB-D cells nor in PLB cells in the presence of indomethacin, indicating that Fc gamma RIIA gene induction is regulated by CREB activation stimulated by the PGE₂-PKA pathway.

D3-006

Collectins and the effect of different N-linked glycan profiles on their antiviral activity

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Surfactant proteins A (SP-A) and D (SP-D) belong to a subgroup of mammalian collagenous Ca²⁺-dependent lectins

known as the collectins. These multimeric glycoproteins are important sugar pattern recognition molecules, involved in the innate immune response against microorganisms. Characterization studies performed on porcine SP-A (pSP-A) and SP-D (pSP-D) revealed that both collectins display unique glycosylation characteristics in their lectin domains, compared to those from other animal species. Porcine SP-D features an N-linked complex type oligosaccharide which is absent in SP-Ds from other species. This glycan moiety appears to be fully and exclusively sialylated with α(2,6)-linked sialic acids (SAs). In contrast, the conserved N-linked carbohydrate present on SP-A is partly sialylated via both α(2,3) and α(2,6) types of linkage. However, pSP-A contains predominantly α(2,3)-linked SAs. Hemagglutination inhibition studies performed with influenza A virus (IAV) illustrated that due to the unique sialylation profile of pSP-A and pSP-D, these proteins show an enhanced anti-IAV activity against various IAV strains. Furthermore, it was shown that the type of SA-linkage plays an important role in IAV-binding and neutralization. Considering the importance of collectin glycosylation for its pathogen neutralizing activity, a mammalian cell expression system is used to produce recombinant pSP-A and pSP-D, as well as various mutants that differ in degree, location(s) and composition (e.g. SA content, type of linkage) of N-linked oligosaccharides. Screening of these recombinant proteins using infectivity neutralization assays allows to determine the functional implications of glycan modifications for the antiviral efficacy of collectins in more detail.

D3-007P

Biochemical characterization of a lectin from the white shrimp *Litopenaeus setiferus* (Crustacea:Decapoda)

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We purified a lectin (LSL) from the *Litopenaeus setiferus* hemolymph by affinity chromatography on stroma from rabbit erythrocytes. LsL is a 290 kDa protein composed by 80 and 52 kDa subunits. LsL is devoid of carbohydrates, constituted mainly by GLX, ASX, GLY and ALA, in minor proportion MET and CYS. Amino acid sequence of LsL predicted from tryptic peptides from each subunit by MALDI-TOF, showing 23 and 22%, respectively, homology with the hemocyanin precursor from *L. vannamei*. CD revealed 49% of beta sheets and 6.1% of alpha helix. LsL agglutinates several sialylated erythrocytes and is Ca²⁺ dependent. Sialylated O-glycosylproteins were powerful than GlcNAc, GalNAc, and Neu5Ac to inhibit hemagglutinating activity. LsL could be considered an I-type lectin, which participate in defense mechanisms in invertebrates.

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D3-008P**Corticotropin-releasing factor (CRF) and the urocortins induce the expression of TLR-4 in macrophages via activation of the transcription factor PU.1**

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Corticotropin-releasing factor (CRF) augments lipopolysaccharide (LPS)-induced macrophage cytokine production. The aim of the present study was to determine the mechanism by which CRF and its related peptides urocortin 1 (UCN1) and urocortin 2 (UCN2) affect LPS-induced cytokine production from macrophages. We examined their role on toll-like receptor 4 (TLR4) expression and PU.1 activation, since LPS exerts its effect via TLR4, the expression of which is primarily regulated by PU.1. For this purpose, the murine macrophage cell line RAW264.7 and primary murine peritoneal macrophages were used. Our data are as follows: exposure of peritoneal macrophages and RAW264.7 cells to CRF, UCN1 or UCN2 induced TLR4 expression as documented by an increase of its transcript and protein levels. To confirm that the effect occurred at the transcriptional level, RAW264.7 cells were transfected with a minimal TLR4 promoter containing 550 bp of the proximal promoter region (containing three PU.1 responsive elements) linked to the luciferase gene. CRF peptides induced nuclear translocation and DNA binding of the transcription factor PU.1. The effects of CRF peptides were inhibited by the CRFR2 antagonist antisauvagine30 but not by the CRFR1 antagonist antalarmin suggesting that the effect was mediated by the former receptor. Finally, CRF peptides blocked the inhibitory effect of LPS on TLR4 expression. In conclusion, our data suggest that in macrophages CRF peptides, through CRFR2, induce the expression of the TLR4 receptor gene via activation of transcription factor PU.1.

D3-009P**Generation and initial characterization of bovine FcRn alpha chain BAC transgenic mice**

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Maternal immunity is exclusively mediated by colostral immunoglobulins in ruminants. The prominent IgG shows subclass specific distribution in the colostrum. Neonatal Fc receptor (FcRn) was cloned and characterized in bovine (Kacs Kovics et al. *J Immunol*, 2000). FcRn mediates both transcytosis of maternal IgG to the fetus or neonate and IgG homeostasis in adults. Recent data indicate that FcRn plays an important role in the IgG transport during colostrum formation in ruminants. In order to better understand how the expression of the FcRn is regulated in the mammary gland through different lactational stages, BAC transgenic mice were created. The 100 kb long BAC clone isolated from a bovine BAC library (Eggen et al., 2001) harbours the bovine FcRn α chain gene and its 20–70 kb long 5' and 3' regulatory regions. Integration of this size of genomic DNA normally ensures high level and position-independent expression in transgenic animals. Microinjection of mice (donor females: FVB/N, recipient females: CD1) was performed with minor

changes in the composition of microinjection buffer (supplied or not with spermine/spermidine). Three independent transgenic mouse lines were created through microinjection. Two of those lines (#14, #19) showed Mendelian pattern of the transgene inheritance, while in the third line (#9) this pattern indicated the presence of two independent integration sites. Transgene copy numbers were found to be 2 and 5 in #14, #19 lines respectively as determined by real time PCR method. Transgenic mice are indistinguishable from their littermates based on their weight and overall health. Bovine FcRn α chain specific mRNA was detected by RT-PCR and Northern analysis in the liver and intestine of hemizygous individuals from all three transgenic lines and in lactating mammary gland of line #14. To reveal if the bovine FcRn α chain is able to form functional receptor, 600 μ g human IgG was injected i.v. into transgenic and control mice and human IgG concentration was monitored in serum samples through 100 h. Preliminary data show extended half life of human IgG, which indicates the presence of functional bovine FcRn in BAC transgenic mice.

D3-010P**Immunostimulating activity of *Mycobacterium phlei* cell extracts**

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Bacillus Calmette–Guerin (BCG) application is the standard treatment for some type of the superficial bladder carcinoma. Despite being an effective therapeutic, pathogenicity and lethal side effects of BCG limits its usage. In order to find less toxic and more potent therapeutic agents for the treatment, researches with *Mycobacterium phlei* are carried away. Cell wall extract of *Mycobacterium phlei* is sufficient for antitumoral activity. It is proposed that this bacteria has direct apoptotic activity in addition to BCG like immunostimulating activity. The aim of this study is to search for the fractions of *Mycobacterium phlei* cell extracts that are effective in inducing TNF- α and IL-12 response in host cells. *Mycobacterium phlei* was grown in Middlebrook 7H9 medium. The cells were disrupted by sonication and several different extracts, such as protein rich, lipid rich, nucleic acid rich were prepared by centrifugation, ethanol, SDS and acetone treatments. These extracts were incubated with human macrophages. TNF- α and IL-12 responses by these fractions were investigated. Several fractions showed TNF- α and IL-12 response, the highest being the extracts rich in cell wall proteins.

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D3-011P**Immobilized C1q induces maturation of human monocyte-derived dendritic cells**

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Dendritic cells (DCs) link innate and adaptive immunity by their ability to present antigens to naive T lymphocytes. During their

development to mature DC expression of receptors required for phagocytosis are down-regulated, while MHCII and CD83 proteins as well as co-stimulatory molecules are up-regulated. Maturation of DC, a decisive step to initiate T-cell activation, is known to be induced by several stimuli, including inflammatory cytokines, microbial products (e.g. LPS) and immobilized IgG. Since immune complexes occurring *in vivo* contain C1q as well, our aim was to study whether this complement protein and its structurally related molecule MBL influence maturation of DCs. Immature monocyte-derived DCs (imMDCs) were generated from human monocytes in the presence of IL-4 and GM-CSF, and on day 5 the cells were transferred to plates coated with C1q or MBL used at 70 µg/ml. As control, LPS was added to the cells. On day 7 MDCs were characterized by expression of various cell membrane molecules, by their cytokine secretion pattern and by the efficiency of cells to activate allogeneic T lymphocytes. To find out whether C1q induce TH1 or TH2 immune response we measured the cytokine production of T cells cocultured with C1q activated imMDCs. We found, that immobilized C1q, but not MBL induces maturation of imMDCs to a similar extent as LPS. Interaction of C1q with imMDCs was strong and decreased with maturation of the cells, while we could not detect any binding of MBL to DC. Treatment of imMDCs with C1q induced NF-κB translocation from the cytoplasm to the nucleus. IL-6, IL-10 and IL-12 secretion and T-cell stimulation by imMDCs cultured on immobilized C1q were almost as high as in the case of LPS-stimulated cells. TNF-α, IL-10 and IFN-γ(were also measured in supernatants of MDC-T cocultures. Our data suggest that interaction of C1q with imMDC generates a TH1-type response.

D3-012P

Polyreactivity of IgG induced after treatment with ferrous ions relates to increased hydrophobicity and plasticity of the antigen-binding site

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Polyreactivity is defined as the ability of a given antibody molecule to bind several structurally unrelated antigens. A fraction of circulating IgG in healthy individuals becomes polyreactive after exposure to certain chemical factors, including low or high pH, high salt concentration and chaotropic agents. Enhanced polyreactivity of IgG does not result from the denaturation of the molecules. We have shown previously that the exposure to ferrous ions of some monoclonal IgG and of polyvalent human IgG for therapeutic use (IVIg) also results in dramatically increased polyreactivity. Here, we aimed at deciphering the molecular mechanisms that underlay the increase in IgG polyreactivity and evaluated its biological relevance. To answer these questions, we have used a monoclonal IgG sensitive to treatment with ferrous ions as well as IVIg. pH scanning analysis and fluorescence spectroscopy demonstrated that the increase in polyreactivity of IgG upon treatment correlates with the appearance of hydrophobic patches on the IgG surface. The hydrophobic effect was found to play a major role in the interaction between treated IgG and their target antigens. Further, kinetic and thermodynamic studies performed using surface plasmon resonance-based technique suggested that enhanced polyreactivity results from an increase in the structural flexibility of the IgG paratope. We pro-

pose that the increased plasticity of the antigen-binding site allows it to accommodate structurally distinct epitopes and accounts for IgG polyreactivity. Polyreactivity may play an important role in innate immunity under physiological conditions as ferrous ions-treated IVIg, were found to protect mice from death in a model of septic shock.

D3-013P

Effect of phenols on K562-mediated NK cell activity

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As one contemplates immunologic strategies to fight cancer, immune integrity in cancer patients needs to be considered. Because in cancer patients immune integrity is disrupted and standard chemotherapies may further diminish critical immune response, the potential of natural factors to modulate immune response is of immense interest. To clarify whether natural antioxidant phenols render K562 human leukemic cells more susceptible to the cytotoxic activity of natural killer (NK) cells, K562 human leukemic cells were pre-incubated with several phenols and afterwards targeted with NK cells isolated from healthy donors at a ratio 1:5. Percentages of apoptotic and necrotic cells were assayed via flow cytometric analysis of annexin V and propidium iodide stained cells. For the morphological assessment, cells were stained with acridine orange and ethidium bromide and examined under a fluorescence microscope. Co-culture of cells for 24 h (control) triggered 86.6 ± 3.1% of K562 to apoptosis and 0.81 ± 4.6% to necrosis. K562 cells pre-treated with gallic acid at 200 µg/ml for 24 h and afterwards co-cultured with NK cells for another 24 h underwent apoptosis at 8.44 ± 4.1% and necrosis at 90.3 ± 3.4%, meaning a remarkable clear increase in the percentage of necrotic K562. On the other hand, flavonol rutin at 50 µg/ml was shown to trigger NK immune response increasing mainly the percentage of apoptotic K562 (97.7 ± 3.3%) and secondary of the necrotic (1.99 ± 3.4%). Percentages of apoptotic and necrotic cells were not altered significantly when pretreating K562 with any of the other phenols tested.

D3-014P

Macrophage migration inhibitory factor (MIF). Enzymatic activity of peritoneal fluids in endometriosis

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The disordered immuno-endocrine regulation has long been recognized as a major feature of diverse reproductive dysfunctional states. Derangement of certain immune functions has also been described as a pathogenetic factor in endometriosis. Ectopic endometrial plaques have recently been reported to aggravate peritoneal macrophage responses including augmented secretion of the cytokine macrophage migration inhibitory factor (MIF). MIF features a peculiar enzymatic activity: phenylpyruvate tautomerase. It is not established at present, whether the enzymatic activity is related to the cytokine function of the protein. Therefore in peritoneal fluid samples obtained during zlaparoscopy from endometriotic and from non-endometriotic patients (total: 47) we have assessed the correlation of the immunoreactivity (duo set capture ELISA by R&D Sciences) with the enzymatic

activity of MIF. Western blots were performed following polyacrylamide gel electrophoresis to analyse MIF immunoreactivity of different polypeptides. Enzymatic activity was measured by a spectrophotometric assay using phenylpyruvate as substrate. Hereby we report that immunoreactivity and enzymatic activities of MIF are not in the expected close correlation concerning the peritoneal fluid. The suspected underlying causes are discussed. Nevertheless, the possible relevance of MIF's contribution to the pathomechanism of endometriosis warrants further investigation with special regard to the involvement of its peculiar enzymatic activity.

D3-015P

Identification of genes induced in virus-infected silkworm (*Bombyx mori*)

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Though cellular reactions like phagocytosis, nodulation and encapsulation and humoral reaction through secretion of antibacterial proteins are known for fungus and bacterial invaders, little is known about insect defence against insect virus infection. To obtain genes related to insect antiviral immunity from *B. mori*, the cDNA library was constructed from nucleopolyhedrovirus (BmNPV)-infected *B. mori*. From the cDNA library, we selected 411 differentially expressed clones, and the 5' ends of the inserts were sequenced to generate ESTs. In this work, 141 unigenes were generated after the assembly of 411 differentially expressed clones ESTs. Of these 141 unigenes, a total of 72% had significant matches to genes from other organisms in the database, whereas 28% of the unigenes had matched in the database. Functional groups of these sequences with matches in database were constructed according to their putative biological function. Three largest categories were protein fate (20%), metabolism (20%), and cellular transport and transport mechanism (14%).

D3-016P

Adenosine deaminase isoenzymes expression in human blood cells

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Adenosine deaminase (ADA, EC 3.5.4.4) deaminates (deoxy) adenosine to (deoxy)inosine. ADA is widely distributed in mammalian tissues. Its role is critical in proliferation, maturation and function of lymphoid cells. Three molecular forms of human ADA are known: small and large isoforms of isoenzyme ADA1 and isoenzyme ADA2. ADA1 and ADA2 differ in kinetic and immunochemical properties and probably are coded by separate genetic locus. ADA2 is a minor component of ADA activity in many tissues but predominates in the blood serum. It seems essential to recognize the blood cell system contributing to regulation of serum ADA isoenzymes. We investigated the ADA isoenzymes producing by human peripheral blood cells *in vitro*. Blood samples ($n = 10$) were analyzed on total ADA, ADA1 and ADA2 activities in serum; they were characteristic for normal blood serum, 10–15, 2–3 and 7–9 U/l, respectively. Peripheral blood mononuclear cells (PBMC) and polymorphonuclear neutrophil granulocytes (PMN)

were isolated according to Boyum and suspended in Hanks balanced saline solution (HBSS). The ADA isoenzymes activity in isolated cells shown rather low ADA1 activity and ADA2 activity forthcoming to zero. The time-dependence of isoenzymes expression by cells incubated in HBSS at 37 °C was studied. The main observations were: (a) all cell types studied produce ADA2 activity with similar kinetics, reaching the peak in 6 h on average (worthy of notice – the time of inflammatory interleukins expression peak at the same experimental conditions); (b) at increasing of one isoenzyme activity, the activity of the other isoenzyme decreases; (c) the time-dependence of isoenzymes expression in cells activated with boiled *Escherichia coli* is identical.

D3-017P

In vitro phenoloxidase activation detected during the development of the Pacific oyster, *Crassostrea gigas* (Thunberg)

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In this study we described the presence of phenoloxidase (PO) activity in the different developmental stages of the Pacific oyster *Crassostrea gigas*. A significant reduction in PO-like activity was observed from the “6 h embryo” stage to the day 11 stage of larvae through to the juvenile stage. The microscopy studies with “6 h embryo” and adult samples confirmed too the presence of PO-like activity observed by spectrophotometry. Various modulators PO-like activity were used to study the triggering of pro-phenoloxidase (pro-PO) activating system of *C. gigas*. The enzyme activation mechanisms appear to differ with the developmental stage: bacterial lipopolysaccharides (LPS) constitute an early elicitor of the pro-PO system during the growth of the oyster, whereas a protease, a purified trypsin (TPCK) triggers proPO-PO system during the final developmental phase of *C. gigas*. Phenoloxidase activity was totally suppressed by PO-specific inhibitors such as β -mercaptoethanol, sodium diethyldithiocarbamate and tropolone. This study demonstrates the selective response of PO-like activity by different elicitors and suggests too that proPO-PO activating system, which is supposed to play an important function in non-self recognition and host immune reactions in oyster, is expressed early in the Pacific oyster, *C. gigas*.

D3-018P

DNA-dependent protein kinase (DNA-PK) regulates V(D)J recombination by RAG2 phosphorylation

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V(D)J recombination is a major process that confers the diversity of antigen receptors in the immune system. This process begins specifically at two recombination signal sequences by forming a synaptic complex with two RAG1 and RAG2 proteins and other proteins such as HMG1. RAG1 and RAG2 initiate double-stranded DNA breaks at the border between coding and signal sequence, resulting in formation of hairpined-coding ends. Then, other double-stranded DNA repair proteins seal two broken coding ends after hairpin opening. According to many previous studies, DNA-dependent protein kinase (DNA-PK) have been specifically suggested its role in V(D)J recombination by involving at the hairpin opening step. Recently, DNA-PK phosphory-

lates Artemis, an exonuclease. The phosphorylation can switch it to an endonuclease that resolves the hairpin structure during the V(D)J recombination process. In this study, we found that DNA-PK catalytic subunit specifically phosphorylates RAG2 protein at the 365th serine residue. Mutation at this serine residue to alanine (S365A) caused about 10-fold decrease of RAG2 phosphorylation by DNA-PK *in vitro*. In addition, RAG2 mutant S365A severely lowers V(D)J recombination activity in cell-based assay. These results suggest that DNA-PK might be involved in V(D)J recombination by modifying RAG2 activity.

D3-019P

Mycoplasma synoviae haemagglutinin induces no production and IL-6 secretion in chicken macrophages

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Mycoplasma synoviae (MS) is the causative agent of chronic respiratory disease and infectious synovitis in chickens and turkeys. The MS haemagglutinin VlhA, an 82 kDa lipoprotein, is a major surface membrane protein and immunogen, involved in the adhesion of MS to host cells. VlhA is post-translationally cleaved into MSPB and MSPA subunits. Lipoprotein MSPB can be found in full-size (45 kDa) and truncated (25 kDa) length fragments. Stimulation of cytokine, chemokine and mitogen secretion by MS cells or membrane fractions that is presumably caused by diacylated N-terminus of lipoproteins has been previously reported. Additionally we have reported that precipitated and non-precipitated MS membrane protein extracts induce NO production and IL-6 secretion in chicken macrophages. With the intention of further specification the activating agent of the membrane protein fractions we used Western Blot transfer of SDS-PAGE separated MS proteins. Bands of 45 and 25 kDa, confirmed as MSPB by specific mAb were excised from the membrane, eluted and precipitated. The single band proteins were used to stimulate macrophages derived from chicken peripheral blood (PBM), for IL-6 secretion analysis, and chicken macrophage cell line MQ-NCSU, for NO production analysis. MSPB proteins (45 and 25 kDa) caused a dose-dependent increase of NO production in MQ-NCSU cells and IL-6 secretion in PBM comparable to the effect of the LPS. The size of the fragments, i.e. full-length or truncated form, had no significant influence on the level of NO production and IL-6 secretions. This indicates that amino acids in the excess of 20 kDa segment at the C-terminus of 45 kDa MSPB do not contribute to the activation of host cells. Our data further stresses the importance of diacylated N-terminus of MSPB for the reaction with the chicken host cell receptors that remains to be detected in further experiments.

D3-020P

Enzyme activity of macrophage migration inhibitory factor (MIF). Inhibitory effect of plant-derived anti-inflammatory compounds

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Better understanding of the pathogenic mechanisms involved in development of chronic inflammatory conditions like rheumatoid arthritis (RA) might lead to more efficient treatments with less side-effect. The cytokine macrophage migration inhibitory factor

(MIF) has recently emerged as a crucial factor in RA pathogenesis. It is debated whether the MIF mediated tautomeric conversion of either phenylpyruvate or of its other phenolic substrates is implicated in the pro-inflammatory action of this cytokine. Traditional herbal remedies have been used for centuries to alleviate inflammatory ailments of many kinds including arthritis. Several of the active ingredients identified are mono- or polyphenol derivatives. In the present study the effect of some anti-inflammatory plant phenols on MIF mediated tautomerism of phenylpyruvate was investigated. Curcumin and caffeic acid were found the most potent inhibitors, exhibiting IC₅₀ values in the submicromolar range in the ketonase assays. Inhibitor potencies of resveratrol and umbelliferon were similar to the values reported earlier for the popular non-steroidal anti-inflammatory drug acetaminophen in the keto-enol conversion. Our results reveal MIF as a possible target for the herbal anti-rheumatic agents.

D3-021P

Proteolytic activity of antibodies from human milk

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Catalytic antibodies (abzymes) were found in blood of patients with bronchial asthma, systemic lupus erythematosus, rheumatoid arthritis, systemic scleroderma, autoimmune thyroiditis. At the same time our data indicate that antibodies isolated from blood of healthy donors and of patients with influenza, pneumonia, tuberculosis, tonsillitis, duodenal ulcer, and some cancers did not exhibit any catalytic activity. During pregnancy and immediately after delivery (i.e. at the beginning of lactation), the women organism is frequently characterized by an immune status similar to that of patients with autoimmune diseases. In addition, lactation is associated with production of catalytically active antibodies with DNase, RNase, ATPase and protein kinase activities in breast milk. Here we have studied in detail the enzyme properties of antibodies: type of proteolytic activity, substrate specificity, pH optimum and KM characterizing the interaction of sIgA with casein, an abundant human milk protein. The results demonstrate that the proteolytic activity is an intrinsic property of Ig from milk. According to a set of stringent criteria, these activities are not due to unknown co-purifying enzymes, and display biochemical properties quite different from all known proteases. Abzymes hydrolyze human and bovine casein but not many other proteins tested. Specific inhibitors of acidic and thiol proteases had little effect on the proteolytic activity of Ab, while specific inhibitors of serine proteases (AEBSF) and metalloproteases (EDTA) significantly inhibited the activity of proteolytic abzymes. The KM value was 7.3×10^{-6} M. Our findings clearly show production of IgGs and IgAs with casein-specific proteolytic activity by the immune systems of clinically healthy mothers.

D3-022P

The effect of carvedilol on respiratory burst of phagocytes *in vitro*

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Superfluous reactive nitrogen and oxygen species generation is implicated in the damage of tissues at sites of inflammation

where activated neutrophils and macrophages are involved. Availability of nitric oxide (NO) scavengers at sites of inflammation may play an essential role in up-regulation of the catalytic activity of inducible nitric oxide synthase (iNOS). Myeloperoxidase (MPO), a major NO scavenger, is a pivotal enzyme involved in leukocyte-mediated host defenses. However, a detailed understanding of the interrelationship between iNOS and MPO at sites of inflammation is lacking. We studied the effect of carvedilol (CAR) [0.1–100 $\mu\text{mol/l}$], a unique cardiovascular drug with antioxidative properties, on respiratory burst and MPO release from isolated human neutrophils stimulated by particle phagocytosis (OZ) or by activation with soluble stimulus (N-formyl-methionyl-leucyl-phenylalanine (FMLP)). CAR dose dependently decreased OZ stimulated superoxide (SO) generation. Wortmannin, a specific inhibitor of 1-phosphatidylinositol 3-kinase, inhibited significantly FMLP stimulated SO generation only. CAR (100 $\mu\text{mol/l}$) with wortmannin (100 nmol/l) decreased SO generation after both stimuli (OZ, FMLP). CAR decreased OZ and FMLP stimulated MPO release dose dependently (1, 10, 100 $\mu\text{mol/l}$). RAW 264.7 cell line (murine macrophages) was used for the study of iNOS expression and NO production. CAR (100 $\mu\text{mol/l}$) completely inhibited the production of nitrites as well as expression of inducible iNOS (Western-blot analysis). Our results suggest that beneficial effect of CAR involves its antioxidative and scavenging activities, its physicochemical properties, as well as interference with signal transfer transduction.

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D3-023P

Role of the calcium ion for electrostatic stability and functional activity of human C1q globular domain

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C1q is the recognition subunit of the classical complement pathway and serves as a connecting link between the innate immunity and the acquired immunity. The intact C1q is a complex molecule, divided to collagen-like and globular domains, the latter containing calcium ion with unknown function. To understand if calcium ion plays a stabilizing role in the heterotrimeric assembly or/and it has some other role in the realization of C1q functional activity, we approached this problem theoretically and experimentally. The availability of the crystal structure of the globular C1q heterotrimer provides the opportunity for calculation of the electrostatic free energy and net charge of the molecule with and without calcium as well as full 3D mapping of the molecular electrostatic potential and its derivatives. On the other hand the availability of well characterized recombinant globular fragments of the three chains allow investigation of the role of the calcium over the interaction between them and the important natural C1q-target molecules – IgG1, IgM and C-reactive protein. A good correlation between the theoretical modeling and the experimental data has been observed. Our results indicate that the calcium ion mainly influences the recognition activity of C1q toward target molecules rather than the electrostatic stability of the heterotrimer. Loss of calcium changes the direction of the electric moment from co-axial to molecular axis (where the puta-

tive CRP-binding site is located) to perpendicular to it (where is IgG-binding site) which can be important for target recognition. We also propose a model for calcium-controlled structural changes in C1q, which are a necessary step in the complement activation.

D3-024P

IL-10 gene deficient mice lack TGF-beta-mediated Smad signaling and TLR2 protein degradation in intestinal epithelial cells after the colonization with colitogenic *Enterococcus faecalis*

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Non-pathogenic enteric bacterial species initiate and perpetuate experimental colitis in interleukin 10 gene deficient mice (IL-10^{-/-}). Bacteria-specific effects on the epithelium are difficult to dissect due to the complex nature of the gut microflora. We showed that IL-10^{-/-} mice compared to wild type mice fail to inhibit pro-inflammatory gene expression in native intestinal epithelial cells after the colonization with colitogenic Gram-positive *Enterococcus faecalis*. Interestingly, pro-inflammatory gene expression was transient after 1 week of *E. faecalis* monoassociation in IEC from wild type mice but persisted after 14 weeks of bacterial colonization in IL-10^{-/-} mice. Accordingly, wild type IEC expressed phosphorylated NF- κ B subunit RelA (p65) and phosphorylated Smad2 only at day 7 after bacterial colonization, whereas *E. faecalis*-monoassociated IL-10^{-/-} mice triggered persistent RelA but no Smad2 phosphorylation in IEC at days 3, 7, 14 and 28. Consistent with the induction of TLR2-mediated RelA phosphorylation and pro-inflammatory gene expression in *E. faecalis*-stimulated cell lines, TLR2 protein expression was absent after day 7 from *E. faecalis*-monoassociated wild type mice but persisted in IL-10^{-/-} IEC. Of note, TGF- β 1-activated Smad signaling was associated with the loss of TLR2 protein expression and the inhibition of NF- κ B-dependent gene expression in IEC lines. In conclusion, *E. faecalis*-monoassociated IL-10^{-/-} but not wild type mice lack protective TGF- β /Smad signaling and fail to inhibit TLR2-mediated pro-inflammatory gene expression in the intestinal epithelium, suggesting a critical role for IL-10 and TGF- β in maintaining normal epithelial cell homeostasis in the interplay with commensal enteric bacteria.

D3-025P

Antimicrobial spectrum determination of the K5 type yeast killer protein, its kinetics of cell killing and formulation studies for industrial applications

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Some yeast strains secrete into the medium polypeptide toxins which are inhibitory to sensitive microbial cells. These yeast strains are termed as killer yeasts and their toxins are designa-

ted as killer proteins or killer toxins. Killer yeasts are classified into 11 typical types (K1–K11) on the basis of killing and immunity reactions. Some killer toxins disrupt cytoplasmic membrane function or inhibit DNA synthesis or stop cell division at G1 phase. Others hydrolyze or inhibit the synthesis of major cell wall component β -1,3-glucans. As mammalian cells lack cell walls research and development of novel highly selective antifungals are mostly focused on the agents which target the components of the fungal cell wall. Studies based on the microbial interactions between *P. anomala* NCYC 434 and human and plant pathogenic fungi showed that this strain has a powerful inhibitory effect against the tested pathogens and thus its killer toxin has been suggested as a novel antifungal agent in the medical field and plant protection. In our previous study for the first time we have characterized the K5 type killer toxin which is responsible for the killing effect of *P. anomala* NCYC 434. Its β -1,3-glucan hydrolyzing activity and stability at pHs and temperatures appropriate for industrial applications highlighted the potential use of this protein as a selective antimycotic agent in the medical field and in plant protection. This work covers the killing spectrum analysis, MIC determinations, kinetics of cell killing studies of the K5 type yeast killer toxin on *Candida* species and dermatophytes which cause human systemic and local fungal infections and plant pathogenic fungi along with its formulation studies.

D3-026P

MOG peptide specific antibody response in the mouse model of multiple sclerosis shows no correlation with the severity of the disease

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Multiple sclerosis (MS) is the most common inflammatory and demyelinating disease of the central nervous system. In both MS and its animal model experimental autoimmune encephalomyelitis (EAE), it is thought that infiltrating CD4+ T cells initiate an inflammatory process and collect other immune effectors to mediate tissue damage. However, the pathophysiology of the disease remains unclear. Our studies are focused on the role of the complement and myelin specific antibodies. Female C57BL/6 mice were immunized with myelin oligodendrocyte glycoprotein (MOG) peptide 35–55 emulsified in complete Freund adjuvant and pertussis toxin. Clinical signs of EAE were rated daily using a standard scale of 0–5. First we investigated the role of *in vivo* de complementation by cobra venom factor (CVF). We found that mice with low complement activity at the time of induction fail to show the symptoms of the disease; however, the mice with normal complement levels develop a severe form of EAE. This study has shown that animals with severe symptoms have a higher level of MOG-specific antibody. Therefore, in the next experiment we have created a group with higher antibody level by injecting MOG peptide into their footpad. Lymph nodes from these animals were used in the T cell proliferation tests. As opposed to our expectations, our results show that mice with high antibody level stay healthy. However, these healthy animals have MOG specific T cells as our proliferation tests show. Serum treatment of the APCs increased the proliferation of the T cells, verifying the effect of the complement. Curiously, the brain-homogenate specific antibody level in all groups was almost the same. To sum up our results we can say that complement has effect on the development of EAE, but it seems it is not through the antibody mediated pathway.

D3-027P

Enterobacterial 38 kDa major outer membrane protein is immunoreactive with human IgG antibody of umbilical cord plasma and sera of children and adults

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The major outer membrane proteins (OMP) called porins in several Gram-negative bacteria are immunologically important components because they are exposed on the cell surface [1]. This feature makes them attractive as potential vaccine candidates and also a convenient carrier for the carbohydrate antigens. In earlier studies we have observed immunogenic and protective properties of OMPs from *Salmonella*, *Shigella* and *Hafnia* strains [2]. Recently it was suggested that in an animal model of shigellosis 38 kDa and three other OMPs are major antigens in the induction of protective immune response [3], thus these particular OMPs could be candidates for the carrier proteins for the construction of vaccines. In order to investigate normal human sera for their reactivity with OMP we have isolated them from *Shigella flexnerii* and several other Gram-negative bacterial strains. The OMP antigens resolved in SDS-PAGE were subjected to immunoblotting with umbilical cord plasma and the collected sera from children and adults with detecting the IgG antibodies. The experiments revealed that IgG of human umbilical cord plasma and adult sera reacted primarily with 38 kDa protein. The similar profile of OMP reactivity was observed for all studied strains except of *Pseudomonas aeruginosa*. This observation prompted us for the determination of the level of reactivity of human sera with this 38 kDa protein. This protein was isolated from *Sh. flexnerii* with preparative SDS-polyacrylamide gel electrophoresis and used in ELISA. The reactivity of this protein with sera was dependent on age, namely the reactivity in infants dropped at 4 months and increased beginning from 12 months to 5 years of age, then reaching the IgG level of adult serum. The results show that the 38 kDa OMP is a major enterobacterial protein recognized by human immune system playing a protective role against enterobacterial infections. This might indicate for its use as a safe carrier in conjugate vaccines.

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D3-028P

Molecular cloning and characterization of a cDNA encoding transferrin homologue from *Spodoptera litura*

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We isolated an anonymous cDNA representing a message that was strongly expressed by injection with *E. coli* in *Spodoptera litura*. The 2306 bp cDNA has an open reading frame of 681

amino acid and a predicted Mw of 75.7 kDa. The cDNA sequence shared high homology with the transferrins so far known, and its deduced peptide had unique features showed in a transferrin, sites of cystein residues and iron binding. The mRNA expression of transferrin was accelerated by injection with *E. coli* in the *Spodoptera litura*. We suggest that the *Spodoptera litura* transferrin plays an important role in self-defense system.

D3-029P

Isolation of an O-glycosidically linked receptor from human T lymphocytes

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We purified an O-glycoprotein by affinity chromatography using the lectin from *Amaranthus leucocarpus*. The receptor (ALLr), is a 70 kDa glycoprotein, constituted mainly by serine, glycine, and glutamic acid; its glycosidic portion contains mainly GalNAc; Gal, NeuAc, Man and GlcNAc were identified at a lower proportion. By ionic strength chromatography, as well as 2D-electrophoresis we identified four isoforms of ALLr. N-terminal is blocked both in the ALLr and its isoforms, therefore tryptic peptides of ALLr, analyzed through MALDI-TOF, showed 54% homology with a DnaK-core molecular chaperone, 47% with human KIAA protein, and 44% with heat shock protein 8. The most frequent phenotype of the CD4 or CD8 ALL+ T cells was CD45RA+ CD27+, suggesting that the glycoprotein recognized by ALL could be considered a phenotypic marker for early activated T cells.

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D3-030P

The receptor for *Mannheimia haemolytica* adhesin from bovine neutrophils

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Mannheimia haemolytica is the main causative agent of enzootic septicemia and respiratory disease in cattle; it contained a 68 kDa GlcNAc-specific adhesin (MhA) which seems to participate in pathogenicity. The adhesin has been purified by affinity chromatography using stroma from rabbit erythrocytes and we tested their cellular specificity as well as their biological effect in bovine leukocytes. MhA induces increased oxidative burst, determined by NBT reduction of bovine receptors in a dose dependent form and its effect was inhibited with 200 mM GlcNAc, other monosaccharides showed no effect on adhesin. The MhA receptor from bovine CD16+ neutrophils (MhANr) was purified by affinity chromatography on MhA-Sepharose. MhANr is an 83 kDa glycoprotein mainly composed by Glx, Asx, Ser, Gly and no cystein. The glycannic portion which corresponds to 25% by weight is mainly composed by NeuAc, GlcNAc, Man, and Gal. The peptide fingerprint was determined on tryptic peptides by MALDI-TOF, indicating homology with meta-avinculin (29%) which is a membrane protein which participates in the cytoskeleton ordering, confirming that MhA participates as pro-inflammatory by activating bovine neutrophils.

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D4 – Protein complexes and modifications in health and disease

D4-001

Protein glycosylation and congenital muscular dystrophy

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Muscular dystrophies are genetic diseases that cause progressive muscle weakness and wasting. The past fifteen years the causative genes of several muscular dystrophies have been identified. Recent data suggest that the aberrant protein glycosylation of a specific glycoprotein, α -dystroglycan, is the primary cause of some forms of congenital muscular dystrophy. Protein O-mannosylation is rare protein modification and is present in a limited number of glycoproteins of brain, nerve and muscle. One of the best-known O-mannosyl-modified glycoprotein is α -dystroglycan, which is a central component of the dystrophin-glycoprotein complex isolated from skeletal muscle membranes. Muscle-eye-brain disease (MEB) and Walker-Warburg syndrome (WWS) are autosomal recessive disorders characterized by congenital muscular dystrophy, ocular abnormalities and lissencephaly. Both are caused by defects of protein O-mannosylation. The *POMGnT1* gene is responsible for MEB and the *POMT1* gene is for WWS.

POMGnT1 is responsible for the formation of GlcNAc β 1-2Man. *POMT1* has protein O-mannosyltransferase activity, but only when it is co-expressed with another homologue *POMT2*. All 13 mutations found in the *POMGnT1* gene of patients with MEB and 7 mutations in the *POMT1* gene of patients with WWS lead to great reduction of respective glycosyltransferase activities. These findings suggest that defective protein O-mannosylation of α -dystroglycan is the common trait of muscle cell degeneration and abnormal brain structure.

D4-002

The use of llama heavy chain antibodies to study heart muscular protein complexes

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Hypertrophic cardiomyopathy (HCM) is the cause of sudden cardiac death in about two of 1000 young people. Morphologic features of HCM include ventricular hypertrophy and enlargement

of the atria. This disease is caused by dominant mutations in genes encoding the proteins of the contractile apparatus. Mutations have been identified in myosin heavy chain (β -MHC), α -tropomyosin, cardiac myosin binding protein C, cardiac troponin T, cardiac troponin I and the myosin light chains 1v and 2v. These proteins are components of large protein complexes and variation in the composition of these complexes and the interactions between the different components might be a cause for these disorders. Therefore we want to use antibody arrays to study the qualitative and quantitative protein variation in these complexes. This study makes use of single chain antibodies, found only in camels and llamas. These so called heavy chain antibodies consist of two heavy chains and are devoid of light chains. The antigen-binding domain of these unusual antibodies consists solely of the variable domain of the heavy chain (VHH). This feature makes these antibodies very suitable for use in phage-display and array technologies, since there is no need for combining heavy and light chains. Our first experiments have made use of atrial myosin heavy chain (AMHC1) and ventricular myosin heavy chain (VMHC1) to screen the antibody phage library. The isolated VHHs were tested in Western blots, ELISA experiments and on serial sections of chicken embryonic hearts and are performing above our expectations. At the moment we are screening the phage library with other purified heart muscular proteins.

D4-003

Dystrophin bolt

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Muscular dystrophies are a group of chronic muscle wasting diseases. Among them there are over 10 kinds of dystrophies, whose responsible molecules are located on the cell membrane. The cell membrane is composed of lipid bilayer that protects the internal environment of the cell and must be kept intact. Therefore, it is protected extracellularly by the basal lamina and intracellularly by the cytoskeletal actin-networks. They can be easily peeled off, if these three layers are not fixed by dystrophin bolt, [Dystrophin = β -dystroglycan (DG) = α -DG]. β -DG is a transmembrane protein. α -DG mediates between β -DG and laminin of the basal lamina. Dystrophin mediates actin and β -DG. Thus, dystrophin bolt transmembranously fixes these three layers by connecting the basal lamina and actin networks. When dystrophin is absent, actin networks may be peeled off mainly by agitation on contraction giving rise to Duchenne muscular dystrophy. When the laminin = α -DG junction has some failure, various congenital muscular dystrophies take place. In addition, the transmembranous sarcoglycan complex is a tetramer that reinforces the dystrophin bolt binding to the DG complex. If anyone of them is absent, whole complex is lost and muscular dystrophy ensues. These four dystrophies are collectively called sarcoglycanopathy. The natures of dystrophin bolt will be detailed in the lecture.

D4-005

Protein phosphatases are molecular constraints on brain plasticity and memory

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Brain plasticity and memory are complex functions that are sustained by dynamic molecular and biochemical processes. These processes engage multiple signaling pathways that ensure the transmission of signals from the cell membrane to the nuclear machinery in neurons. Protein kinases and phosphatases are major

components of these cascades that control the activity of neurotransmitter receptors, signaling proteins, transcription and translation factors, etc., by phosphorylation/dephosphorylation. Kinases and phosphatases act in a concerted and balanced fashion and protein kinases generally positively modulate signaling, while protein phosphatases negatively modulate signaling. To challenge this view, we took a transgenic approach and altered the activity of two Ser/Thr protein phosphatases, the Ca^{2+} /calmodulin-dependent phosphatase calcineurin (CN) and the protein phosphatase 1 (PP1) in the adult mouse brain. We evaluated the constraint potential of CN and PP1 on brain plasticity and memory in the resulting mutant mice. These studies showed that rising CN activity in the brain decreases several forms of plasticity such as long-term potentiation in the hippocampus and ocular dominance plasticity in the visual cortex. It concomitantly weakens different forms of learning and memory. Conversely, a decrease in CN or PP1 facilitates plasticity in the hippocampus and promotes cognitive performance. Therefore CN and PP1 appear as natural constraints on learning and memory that must be relieved for efficient processing and storage of information.

D4-006

Laforin, a human dual-specificity protein phosphatase with a starch-binding domain.

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Mutations in the EPM2A gene cause lafora disease, a fatal progressive myoclonus epilepsy characterized by the presence of numerous polyglucosan inclusion bodies in nervous tissues. Computer sequence analysis of the gene product (laforin), suggested two domains: an N-terminal starch-binding domain (CBM20), found for the first time in a human protein and a C-terminal dual specificity phosphatase catalytic domain (DSPc). The gene was cloned from human muscle cDNA. GST-laforin co-purified with GroEL and aggregated easily. GST could not be cleaved off by thrombin. His-tagged laforin required a detergent (CHAPS) for its purification. Further studies were performed on the latter construct. Ultracentrifugation studies showed the protein binds to glycogen. It also binds to granular starch. The catalytic activity was tested with p-nitrophenyl phosphate and O-methylfluorescein phosphate (OMFP), two synthetic substrates. Laforin exhibited a better efficiency toward OMFP, indicating that laforin bears a DSP function. Neither glycogen nor smaller sugars affected the laforin catalytic activity. This is in contrast with MAP kinases phosphatases (MKP). Indeed the laforin catalytic domain is homologous to that of MKPs but CBM20 is different from their regulatory domain. For MKPs, the catalytic activity is increased, when the regulatory domain binds to their substrates. Thus it appears that the role of the laforin CBM20 domain is to target the protein to glycogen in the cells. In conclusion laforin may play a role in glycogen metabolism, which has to be elucidated.

D4-007

Proteolytic processing of dystroglycan in muscular diseases

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Alpha-dystroglycan is a cell surface peripheral membrane protein, which binds to the extracellular matrix (ECM), while beta-dys-

troglycan is a type I integral membrane protein, which anchors alpha-dystroglycan to the cell membrane via the N-terminal extracellular domain. The complex composed of alpha- and beta-dystroglycan is called the dystroglycan complex. Here we report a matrix metalloproteinase (MMP) activity that disrupts the dystroglycan complex by cleaving the extracellular domain of beta-dystroglycan. This MMP creates a characteristic 30 kDa fragment of beta-dystroglycan that is detected by the monoclonal antibody directed against the C-terminus of beta-dystroglycan. We also show that the 30 kDa proteolytic fragment of beta-dystroglycan is increased specifically in the skeletal and cardiac muscles of cardiomyopathic hamsters, the model animals of sarcoglycanopathy (LGMD2C, D, E and F) and that this results in the disruption of the link between the ECM and cell membrane via the dystroglycan complex. Finally, we show that the 30 kDa proteolytic fragment of beta-dystroglycan is increased significantly in the biopsied skeletal muscles of the patients with sarcoglycanopathy and Duchenne muscular dystrophy (DMD), but not other muscular diseases such as Becker muscular dystrophy, Fukuyama congenital muscular dystrophy, Miyoshi myopathy, LGMD2A, facioscapulohumeral muscular dystrophy, myotonic dystrophy and dermatomyositis/polymyositis. These results indicate that the proteolytic processing of beta-dystroglycan by MMP contributes to skeletal muscle degeneration by disrupting the link between the ECM and cell membrane in sarcoglycanopathy and DMD.

D4-008P **Ionizing irradiation influence on protein tyrosine phosphatase activity in lymphoid cells**

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The protein tyrosine phosphatases (PTP) are detailed now, as the important functional part of lymphoid tissue promoting interplay of single messenger cascade components in complete system of immune response and in that way creating the possibility for the humoral and cell immunity reaction realization. CD45, the PTP of lymphocytes, was purified and its catalytic features were evaluated. It was shown that ionizing irradiation influence caused reduction of V max accompanied with affinity increasing in case of paranitrophenylphosphate. The decreasing of dephosphorylating ability and affinity with enzyme and substrate molecule was established, when the phosphotyrosine was applied. Thus, irradiation influence leads to increasing unspecific enzymatic activity accompanied with decreasing of dephosphorylating ability in case of more specific substrate. The study of co-operativity between two of phosphatase domains shows that it grows under conditions of total exposure to radiation of rats. The inhibitor analysis of fermentative kinetic confirms that ionizing radiation influence caused changes in the species of inhibition: competitive type turn into mixed one. Also the levels of PTP activities in rat spleen and thymus lymphocytes after mitogen pre-incubation were investigated to evaluate the participation of these enzymes in immune response forming after radiation treatment. It was shown that the exposure to radiation of rats caused the decreasing of thymus, but not spleen PTP involvement in the antigen stimulated immune response development. Taking together, these data allow us to conjecture that changes in PTP activity levels involved in misbalance of immunity response forming in lymphoid cells under conditions of total exposure to radiation.

D4-009P

Adhesion proteins in myoblasts fusion

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Satellite cells (also called adult myoblasts) are myogenic precursor cells localized between the basal membrane and the sarcolemma in skeletal muscles of adult animals. They participate in post-natal growth and regeneration of skeletal muscles. Satellite cells, dissociated from muscle, are able to grow in culture and fuse to form multinucleate myotubes. There are some adhesion proteins that are postulated to play a role in myogenesis. We examined the participation of syndecan-4, integrin alpha3 and beta1 subunit and ADAM12 in myoblast differentiation. These proteins are transmembrane cell adhesion molecules that transduce signals to cells, link cells to extracellular matrix and to other cells. We showed that integrin alpha3 subunit and syndecan-4 were expressed in quiescent satellite cells and activated myoblasts. We found that syndecan-4 played a key role in myoblasts adhesion. We noticed that increase in protein and mRNA encoding integrin alpha3 subunit, integrin beta1 subunit and ADAM12 accompanied myoblasts differentiation. Using double immunofluorescence and immunoprecipitation experiments, we demonstrate that the complex alpha3beta1/ADAM12 is probably involved in the process of myoblasts fusion. Additionally, antibody against the extracellular domain of integrin alpha3beta1, inhibited myoblasts fusion. Taken together, these data demonstrate that syndecan-4, integrin alpha3beta1 and ADAM12 may contribute myoblasts adhesion and fusion.

D4-010P

Regulation of HIF-1alpha activity in airway smooth muscle cells: the role of cobalt and serum

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Hypoxia inducible factor 1alpha (HIF-1alpha) is the regulatory subunit of HIF-1, the transcriptional activator and key mediator of the cellular response to hypoxia. Induction of HIF-1 by hypoxia is predominantly achieved via inhibition of HIF-1alpha proline hydroxylation and subsequent protein stabilization. However, activation of HIF-1 can also occur under normoxic conditions by exposure to heavy metals, treatment with growth factors or oncogenic transformation. The mechanism of HIF-1 induction in these cases is not always known but appears to involve both transcription and translation dependent mechanisms. In order to understand the role of HIF-1 in the physiology of the respiratory tract and its response to hypoxic conditions, we are studying the induction and activation mechanisms of HIF-1alpha in primary cell cultures of airway smooth muscle (ASM) cells derived from rabbit trachea. We have shown that exposure to low oxygen concentration as well as to the toxic metal cobalt can cause a rapid increase of the intracellular levels of HIF-1alpha, which is detected predominantly inside the nucleus. The same effect is also observed under normoxic conditions, when serum is re-added to ASM cells that undergo starvation-dependent differentiation. Using specific inhibitors we have shown that induction of HIF-1alpha by cobalt depends on active protein synthesis and involves the phosphatidylinositol 3-kinase pathway and the production of reactive oxygen species. By a

similar approach we are currently investigating the serum-induced up-regulation of HIF-1 α . In addition, we have developed an *in vitro* system using bacterially expressed human HIF-1 α in order to identify ASM cell factors that affect its DNA binding activity.

D4-011P

New and novel α -glucosidase inhibitors evaluated from the fungal metabolites of tibolone for the management of type II diabetes

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Alpha-glucosidase enzyme inhibitors (AGIs) are one of the approaches to control the blood sugar levels for type-2 diabetes. Diabetes mellitus is occurred due to the deficiency in production of insulin by the pancreas. According to the latest WHO report, there are 177 million people who are suffering from this disease. α -Glucosidase is a membrane bound enzyme at the epithelium of the small intestine that catalyzes the cleavage of glucose from disaccharide. α -Glucosidase enzyme inhibitors act by suppressing the digestion process of dietary carbohydrates. AGIs are given with meals and they function by slowing the breakdown of the complex sugars into glucose. This cause a delay in glucose absorption and lower blood sugar levels, following meals. The AGIs may be used alone or in combination with other medications for diabetes. Inhibition of α -glucosidases causes abnormal functionality of glycoproteins because of incomplete modification of glycans. Suppressions of this process is involve expected for antiviral activity and decreasing of growth rate of the tumor. Tibolone (1) is a synthetic steroid that has progestrogenic and androgenic properties as well as oestrogenic effects. Tibolone and microbial transformed products of tibolone have been investigated against α -glucosidase enzymes. Tibolone yielded thirteen metabolites. Thus metabolites 2, 3, 7, 8, 10, 11, 12, 13 and 14 are the main metabolites obtained in these fermentations. Hydroxy groups situated at C-6 position as metabolites 2, 10, 11 and 13 showed pronounced inhibitory activity against α -glucosidase enzymes

D4-012P

New amyloid-forming proteins

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Amyloid fibrils are formed by proteins or their peptides in the result of a conformational transition from alpha-helix into beta-sheet structure. Despite the different nature of proteins-precursors, their amyloids have common properties: beta-pleated sheet structure with individual beta-sheets oriented parallel to the main axes; insolubility *in vivo*; specific luminescence in the presence of Congo-red and thyoflavin-T. Amyloid deposits, formed by different proteins are observed in different diseases such as

myositis, myocarditis, cardiomyopathies and others. Here we demonstrate that sarcomeric cytoskeletal proteins of titin family (X-, C-, H-proteins) of rabbit skeletal muscles are capable to form amyloid fibrils *in vitro*. These proteins already contain ~90% of beta-sheet structure necessary for formation of amyloids. The amyloid nature of fibrils formed by X-, C-, H-proteins was confirmed by electron, polarization (with Congo-red) and fluorescence (with thyoflavin-T) microscopy. The change of absorption spectrum of Congo-red and the increase of fluorescence intensity of thyoflavin-T in the presence of the fibrils was also demonstrated. The destructive effect of antibiotics tetracycline and actinomycin on amyloids of sarcomeric cytoskeletal proteins has been found. As X-, C-, H-proteins form amyloids easily *in vitro*, there is a danger of fast growth their amyloid deposits *in vivo*. Taking into consideration common properties of amyloids formed by different proteins, our results clear the ways for conducting by amyloidogenesis in human organs and tissues.

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D4-013P

Changes in the isoform composition of myosin light chains upon recovery of mammal heart activity after hibernation: the clue for understanding of molecular mechanisms development of human cardiomyopathies

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In norm, cardiac myosin has specific isoforms of light chains (LCs) in atria (ALC1, ALC2) and ventricles (VLC1, VLC2). We have at the first time registered the appearance of 30–70% ALC1 in ventricles at early stages of dilated cardiomyopathy (DCM) (Akopova et al., 1998; Khalina et al., 2002). At terminal stage of DCM, similar exchanges are not observed. For elucidating the physiological importance of such a change of LC1s composition in DCM, we used the hibernation of ground squirrels as a natural model of reversible suppression of heart function. We have revealed the changes in LC1s isoform composition of ground squirrel myocardium at the different stages of physiological activity of the animal. In ventricles of awaking animals we have found the appearance of ALC1 up to 30%. These isoform changes lead to the increase of actin-activated ATPase activity of myosin and rapid recovery of heart function. In atrium of hibernating squirrels we revealed 30–60% VLC1, not typical for atria of active animals. As enzymatic activity of atrial myosin higher than that of ventricular one, the replacement of ALC1 by VLC1 upon hibernation (like situation in tetralogy of Fallot) may be aimed at reversible decrease of contractile activity that is necessary in this period. Thus the comparative investigations of hibernation and cardiac diseases of different etiology help to understand not only molecular mechanisms of pathology, but also to choose the approach for their diagnostics and correction. **Acknowledgment:** This work was supported by RFBR grants 04-04-48599, 04-04-97305 and grant "Universities of Russia" 1917-05.

D4-014P

Study on criteria of reversibility of the changes in cardiac titin of ground squirrels upon hibernation: new approach for estimating the stage of human cardiac diseases

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By the use of SDS-electrophoresis in agarose-strengthened 2–2.3% polyacrylamide gels the increase by 12–15% in the content of N2BA (long) titin isoform relative to that of N2B (short) titin isoform in left ventricle and atrium of hibernating ground squirrels (*Citellus undulatus*) has been revealed. It is known that the expression of titin isoforms with different extensible regions

represents the mechanism of regulation of myocardium stiffness. The increase of portion of N2BA titin isoform (with longer extensible region) in myocardium of hibernating ground squirrel will lead to the decrease in its stiffness. Such an adaptation must make for the increase of myocardium extensibility and decrease of heart rate that correlates with the available data. Similar increase of N2BA extent in left ventricle of human heart was also revealed at the first stages of dilated cardiomyopathy (Makarenko et al., 2004). The data received upon hibernation prove adaptive nature of this increase. Thus the criteria of reversibility of titin composition changes determined from nature model can be used for estimating the stages of development of dilated cardiomyopathy and other cardiac diseases and will favor the choice of correct approach to their treatment.

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