



EMBO Young Scientists Forum

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EMBO Young Scientists Forum

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Faculty of Science, Zagreb University, Croatia

June 15-17, 2009.

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Abstracts of the Posters

Tobramycin as a signal molecule in *Pseudomonas aeruginosa*

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Aminoglycosides are clinically important antibiotics that have been widely used to treat chronic bacterial infections in the lungs of cystic fibrosis (CF) patients. One of aminoglycosides is tobramycin, produced by the bacterium *Streptomyces tenebrarius*, and is commonly used for its effectiveness against opportunistic CF infections of *Pseudomonas aeruginosa*. Although antibiotics are thought to be small molecules having therapeutic activity in killing or inhibiting microbial growth, few reports have shown that they can act as a small signaling molecules involved in cell-cell communication. Like many other Gram-negative bacteria, *P. aeruginosa* utilizes cell-cell communication systems that rely on diffusible N-acylhomoserine lactone (AHL) signal molecules to monitor the size of population in a process known as quorum sensing (QS).

In our studies we provide evidence that sub-inhibitory concentrations of tobramycin affect directly QS in an environmental isolate of *Pseudomonas aeruginosa*.

We determined the minimal inhibitory concentration of tobramycin in liquid and solid media (LB and M8) for *P. aeruginosa* and also the highest sub-inhibitory concentration which does not affect growth, total protein levels and rates of translation. We have determined that the QS regulated swarming phenotype is affected by sub-inhibitory concentrations of tobramycin. In fact it was established that sub-inhibitory concentrations of tobramycin decrease the levels of one of the two N-acylhomoserine lactone (AHL) quorum sensing (QS) signalling molecules produced by *P. aeruginosa*. It was therefore concluded that tobramycin can act as a signal molecules and regulate QS in *Pseudomonas aeruginosa*.

Results of this study indicate that aminoglycosides can behave as signal molecules at sub-lethal concentrations. These results can be important in the design of appropriate antibiotic therapies of *P. aeruginosa* chronic infections in CF patients.

Immunochemical Characterization of Na⁺-d-Glucose Cotransporters SGLT1 and SGLT2 in Human Kidneys, Small intestine and Liver

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Introduction. In the mammalian nephron, a high affinity/low capacity SGLT1 (SLC5A1) and a low affinity/high capacity SGLT2 (SLC5A2) have been identified as the major mediators of Na⁺-dependent hexose reabsorption. Our immunochemical studies in rats have shown the presence of both transporters in various organs and strong female-dominant sex differences in their expression in proximal tubules (PT) (Balen et al.: *Am. J. Physiol. Cell. Physiol.*, 295:475-489, 2008; and unpublished data). Both SGLTs exhibit 59% homology and some functional and localizational differences. Functional studies in renal tubules and in isolated membranes have shown that SGLT1 transports equally well galactose and glucose, and has been localized to the brush-border membrane (BBM) of PT and small intestine (SI), while SGLT2 transports glucose much better, and has been detected in the BBM of PT. Due to lack of specific antibodies, a detailed immunolocalization of SGLT1 and SGLT2 in the human kidneys (K) and other organs has not been performed.

Materials and Methods. A novel, highly specific rabbit-raised polyclonal antibodies against the human SGLT1 (hSGLT1) and SGLT2 (hSGLT2) proteins were used to study their expression in kidneys (K), SI (jejunum), and liver (L) in humans of both sexes by Western blotting (WB) in isolated total cell membranes (TCM) and by immunocytochemistry (IC) in tissue cryosections.

Results. In WB of the K and SI membranes, the hSGLT1 protein was identified as the 75 (K)-80 (SI) kDa band, while the hSGLT2 protein appeared as the 75 kDa band in the K membranes, which was not detected in the SI membranes. The WB studies in TCM from the K cortex (CO) and outer stripe (OS) revealed the presence of zonal (hSGLT1: CO < OS; hSGLT2: CO > OS) but absence of sex differences in the expression of both hSGLTs. By IC in K, hSGLT1 was restricted to the BBM of PT S3 segments in the OS and medullary rays. In SI, hSGLT1 was detected in: a) BBM and subapical vesicles of absorptive cells, b) apical membrane of crypt epithelium, and c) sporadic individual cells in villous epithelium. A significant hSGLT1-positive staining was also observed in the apical domain of bile ducts in L. Prominent hSGLT2-specific staining was localized to the BBM of PT S1/S2 segments in the K CO, and to the apical domain and/or intracellular organelles in individual, enteroendocrine-type cells scattered among absorptive and crypt cells. In L, no hSGLT2-staining was detected.

Conclusion. Our data confirm that hSGLT1 is critically involved in glucose and galactose absorption in SI, and for reabsorption of these monosaccharides in the renal PT S3 segments and liver bile ducts, while hSGLT2 is mainly engaged in glucose reabsorption in the renal PT S1/S2 segments. Both hSGLTs may contribute to regulation of glucose absorption via the enteroendocrine-type cells in SI.

Key words: enteroendocrine cells, immunoblotting, immunolocalization, glucose transporters, nephron, proximal tubule, sex differences

Correlation of Age and Serum Dipeptidyl Peptidase IV (DPP IV/CD26) Activity in Patients with Inflammatory Bowel Diseases

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Crohn's disease (CD) and ulcerative colitis (UC) are the best known forms of inflammatory bowel diseases (IBD). Their etiology is still unclear, but recent studies indicated the involvement of the immune system in the pathogenesis. Dipeptidyl peptidase IV (DPP IV/CD26) is a membrane-bound multifunctional glycoprotein, acting as a proteolytic molecule, receptor, binding and costimulatory molecule. The proteolytic cleavage of the membrane bound DPP IV results in a soluble form that migrates in the plasma. It has previously been shown that the DPP IV could play a significant role in the pathophysiology of IBD. The aim of this study was to determine the influence of patient's age on the serum DPP IV/CD26 activity in patients affected with IBD. The research was performed on 93 patients, divided in 2 groups: 31 young patients (mean age 13,8 +/- 1,7 years, 24 with CD and 7 with UC) and 62 adult patients (age 42,7 +/- 14,4 years, 38 with CD and 24 with UC). The control group included 111 healthy blood donors: 46 children (age 13,8 +/- 2,8 years) and 65 adults (age 41,6 +/- 12,1 years). Serum DPP IV/CD26 activities in both young and adult patients with IBD were found to be statistically significantly decreased when compared to their healthy controls. Values correlated inversely with the disease severity for both CD and UC. When comparing serum DPP IV/CD26 activities between young and adult patients with IBD, but even between young and adult healthy controls, it was noticed that serum DPP IV/CD26 activity decreases statistically significantly with age. The results of this study show that serum DPP IV/CD26 could be useful as an available, non-invasive marker in the diagnosis of the disease activity. This research also shows that age-related standard values should be undoubtedly established in clinical laboratory practice because of the age-dependent decrease in serum DPP IV/CD26 activity.

Amino acid residues important for catalytic activity of *Streptomyces rimosus* lipase

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Extracellular lipase from *Streptomyces rimosus* – SrL (Q93MW7) belongs to the GDSL family of lipolytic enzymes. Recently, SrL and many other GDSL enzymes were further classified as SGNH hydrolases due to the presence of four invariant and functionally important residues: serine (S), glycine (G), asparagine (N), and histidine (H) placed in the conserved motifs I, II, III and V, respectively. Catalytic triad consists of serine, histidine and often aspartic acid that is positioned three residues upstream of histidine. Glycine and asparagine, together with catalytic serine, serve as proton donors to the oxyanion hole. Although SGNH enzymes have a diverse range of hydrolytic functions and could be used in many industrial applications, there is still much to be learned about their structural characteristics and enzymatic mechanisms.

The aim of this work was to identify amino acid residues essential for catalytic activity of SrL. The structure of this enzyme has not been solved. Based on the protein sequence alignment, it was predicted that catalytic triad was formed of Ser10 and His216, and Asp83 or Asn213 as the third residue. Gly54 and Asn82 were predicted to be involved in forming an oxyanion hole. Site-directed mutagenesis has been applied to obtain enzymes with following mutations: Ser10Ala, His216Ala, Asp83Ala, Asn213Ala, Asn213Asp, Gly54Ala and Asn82Ala.

Purified proteins were analyzed by CD spectroscopy and it was shown that none of the mutations caused significant changes in secondary structure. Thus, we have concluded that differences in specific activity of mutated enzymes could be assigned to the specific mutation of particular amino acid residue. The activities of wild type enzyme and mutants toward standard lipase substrate, p-nitrophenyl palmitate (pNPP), were measured. It was shown that Ser10Ala and His216Ala have almost completely lost pNPP hydrolyzing activity (>2500-fold lower activity was observed), thus confirming their crucial role in catalysis. Further, Gly54Ala and Asn82Ala mutants had residual activity of 58% and 2%, respectively. Asp83 was ruled out as a third catalytic triad residue since Asp83Ala mutant showed activity similar to the wild type lipase. Our results pointed out that the third catalytic residue is Asn213, since Asn213Ala mutant displayed 57% of the wild type activity. This is interesting since the catalytic triad usually consists of Ser, His and an acidic residue. When this asparagine was replaced by aspartate (Asn213Asp), the activity increased to 76% but it still did not reach the full activity of the wild type.

Proflavin: from Astrophysics to Astrobiology

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Proflavin, C₁₃H₁₁N₃ (3,6-diaminoacridine), is well known in biology and chemistry. However, our interest in this material has started in the fields of astrophysics and theoretical condensed matter physics.

The diffuse interstellar bands were discovered ninety years ago as absorption spectral structures from low-density interstellar clouds. Up to now astrophysicists have been measured about 300 diffuse bands distributed mostly in the visible region of the spectrum. Many different carriers of diffuse interstellar bands (such as processes on the surfaces of astrophysical dust grains, or molecules and nanoparticles made of various materials), have been suggested and completely or partially rejected. We study proflavin and related molecules as possible carriers of the diffuse interstellar bands. Proflavin has been also suggested as a molecular «midwife» which increased the rate of DNA and RNA formation in an early stage of development of biological matter on Earth.

The time dependent density functional theory is a method which has been developed as an advancement of the time dependent Schrödinger equation in the quantum physics. Using computational algorithms within this theory we study optical properties of proflavin, its cation and anion. Our results for a neutral molecule in vacuum show a good agreement with laboratory measurements of the optical spectrum of proflavin dissolved in water and at least one line in the diffuse interstellar bands.

Multidrug efflux transporters limit accumulation of inorganic, but not organic, mercury in sea urchin embryos

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In this study we examined the role of multidrug efflux transport in differential accumulation and potency of inorganic (HgCl_2) and organic (CH_3HgCl) mercury in sea urchin (*Strongylocentrotus purpuratus*) embryos. To quantify mercury toxicity we assessed the effect of HgCl_2 and CH_3HgCl on cell division. Our assay detects the incidence of interference with progression through the first cell cycle, which occurs primarily through the effects of mercury on spindles (microtubules) in metaphase and anaphase. We found that the EC_{50} concentrations for interference with mitosis are 610 nM for HgCl_2 and 124 nM for CH_3HgCl . Pharmacological inhibition of MRP/ABCC-type multidrug efflux activity significantly increases the anti-mitotic potency of inorganic mercury, but has virtually no effect on the potency of organic mercury - partial inhibition of the MRP mediated efflux activity with 5 μM MK571, a model inhibitor of MRP efflux transporters, increased the toxicity of HgCl_2 2.8-fold. Similarly, inhibition of these transporters increased intracellular accumulation of HgCl_2 but has no effect on accumulation on CH_3HgCl - 5 μM MK571 caused 2-fold and 20 μM MK571 caused 4-fold increase in intracellular mercury accumulation. Our results point to the MRP mediated efflux transport as an important biological determinant of mercury accumulation and subsequent toxic potency in aquatic organisms.

The potential role of Cys (Cys 139) in the activity of BrILL2, an auxin amidohydrolase from *Brassica rapa* L.

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Auxins are plant hormones involved in many aspects of growth and development, from cell division and elongation to the formation of buds, roots, and fruit. Auxins exist as free auxins (active form) and conjugated with amino acids (storage forms). One of the ways plants control internal levels of auxins is through reversible formation of auxin conjugates. Auxin amidohydrolases are a group of enzymes which hydrolyze the amide bond of conjugated auxins releasing free active auxins. Here we investigate the auxin-amidohydrolase from Chinese cabbage (*Brassica rapa* L.), BrILL2. It is named thus because it shows homology with ILL2, an auxin-amidohydrolase previously identified in *Arabidopsis thaliana*. BrILL2 is a metalloenzyme constituted of 444 amino acids and around 48 kDa in size. According to prediction programs that analyze the amino acid sequence of proteins, this enzyme is probably located in the endoplasmatic reticulum. It hydrolyzes alanine (Ala) conjugates of indole-3-propionic acid (IPA) as a preferable substrate. This enzyme includes 2 Cys which are highly conserved in auxin amidohydrolases through different plant species. One of them (Cys 139) might be substantial for enzyme activity. To obtain enough protein for experiments we used the previously cloned gene for BrILL2 in the expression vector pTrcHis2-Topo which comprises a his-tag. Furthermore, the mutant BrILL2 C139S was obtained by using the QuickChange II XL Site-Directed Mutagenesis kit (Stratagene) and a correspondig pair of primers. The plasmids (wt and mutant) were inserted into *E. coli* cells, strain BL21(DE3)RIL+ by electroporation. Protein overexpression was induced by 0.5 mM IPTG. Proteins were purified by affinity chromatography using Ni-complex linked Sepharose. The enzymatic activities of purified BrILL2, and the mutant BrILL2 C139S were tested. We examined the presence of various reduction agents: DTT, β -mercaptoethanol, reduced glutathione, ascorbic acid, and Cys on enzyme activity. Furthermore enzymes were treated with H₂O₂, and J-acetamide, and, the changes were monitored by SDS-PAGE and by comparing hydrolytic activity with that of the non-mutagenized protein. Progress of the cleavage reaction was monitored by HPLC.

Bioinformatics analyses of BrILL2 were mostly performed by using on line ExPasy programs (<http://www.expasy.ch/>). The 3D structure of BrILL2 was modeled by the program PyMOL using the X-ray structure of IAA-aminoacid hydrolase from *Arabidopsis thaliana* (pdb: 1xmb).

Detection of gibberellin-20-oxidase in Christmas rose (*Helleborus niger* L.) tissues in vitro

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The gibberellins (GA) are plant hormones that participate in many aspects of plant development, including seed germination, flowering, fruit set, and shoot elongation. Such a complex network of plant development and growth processes in which GAs are involved reveals a highly organized regulation of GA levels via GA metabolism. The later steps in GA biosynthesis pathways are catalyzed by a group of enzymes called soluble 2-oxoglutarate-dependent dioxygenases (2ODDs). By acting on the GA skeleton at the 20, 3 β or 2 β positions; 2ODDs can lead either to the formation of the biologically active GAs or to their inactivation. The enzyme gibberellin-20-oxidase (GA 20-ox) catalyzes the final step in the formation of bioactive GAs by removing carbon-20 through its oxidation, thereby enabling formation of the bioactive C19 structure.

In this study, a partial GA 20-oxidase cDNA was generated from Christmas rose (*Helleborus niger* L.) seeds by using degenerative oligonucleotide primers, designed on the basis of nucleotide sequences from conserved regions of related enzymes in other plant species. Furthermore, GA 20-ox cDNA was cloned into a pRSET expression plasmid, and the recombinant GA 20-ox protein was produced in competent *E. coli* BL21(DE3)RIL+ strain. Inclusion bodies (containing GA 20-oxidase) were isolated and separated by SDS-PAGE. The protein band corresponding to GA 20-oxidase was cut out of the gel and used for custom service production of polyclonal antibodies by immunization of a rabbit. The obtained antibodies were purified and used for western blot analysis in order to screen tissues of diverse developmental stages of Christmas rose flower for the presence of GA 20-ox. Soluble proteins were extracted from Christmas rose seeds and such extracts were used to immunoprecipitate the GA 20-ox protein with the same polyclonal antibodies. The acquired protein bands which most likely correspond to our GA 20-ox were excised from the gels and prepared for further peptide sequencing by mass spectrometry. Bioinformatics analyses of GA 20-oxidase were mostly performed by using online ExPasy programs (<http://www.expasy.ch/>).

Effect of p27 on Cell Cycle Regulation and Apoptosis Induction in Lymphomas

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p27Kip1 is a cyclin-dependent kinase inhibitor that binds cyclin-CDK complexes and causes cell cycle arrest in the G1 phase. p27Kip1 is also a mediator of apoptosis. Studies have shown that changes in p27Kip1 intracellular expression and localization are associated with disruption of the cell cycle and the development of malignant tumors. In leukemias and lymphomas, p27Kip1 is often found exclusively in cytoplasm where it can not exhibit its cell cycle inhibitory function. This mislocalization is due to nuclear sequestration by D type cyclin/CDK complexes, nuclear cytoplasmic transport by JAB1 and/or cytoplasmic retention mediated by AKT-dependent phosphorylation.

In our research we transduced p27Kip1 into Raji lymphatic cell line in order to explore the effect of its overexpression on proliferation and apoptosis. Transduction was done using TAT peptide, derived from HIV virus that enables macromolecules to cross cell membrane. In the experiments we used p27Kip1 wild type, p27Kip1 with point mutations in cyclin and cdk binding domains and N terminal part of p27Kip1 protein. The proliferation of Raji cells was estimated using WST test. Influence of p27Kip1 on G1 arrest and presence of Annexin V positive cells was assessed using flow cytometry, after PI-only or PI with Annexin V-FITC staining. Changes in expression of various proteins involved in the regulation of cell cycle and apoptosis were examined using western blotting.

Transduction of p27 variants into the investigated cell line lead to decrease in proliferation and increase of Annexin V binding. Furthermore, we found changes in the expression of AIF, cytochrome c and other proteins involved in cell cycle control and induction of apoptosis.

The results have shown that the influence of transduced p27 proteins depended on the variant of protein. Extracellular p27 induced apoptosis in examined cell line and according to our data it seems that multiple apoptotic pathways are involved. Modulation of p27 expression could be a good candidate for targeted tumor therapy.

Plasmid losing and chromosome rearrangement of *Lactobacillus brevis* L62 during long-term starvation

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Bacteria in a natural environment spend the most of their time in a state of starvation. In order to survive, cells have developed different strategies and mechanisms that facilitate survival during prolonged period of starvation. When faced with starvation some bacteria respond by sporulation, others by undergoing significant physiological, morphological and genetic changes. Stressful environments can result in selection for mutants that express the growth advantage in scantily condition. Growth advantage in stationary phase (GASP) is the term used to describe this phenomenon. It has been observed in gram-negative and gram-positive bacteria. We investigated the survival response of *Lactobacillus brevis* L62 during 10 days of starvation in complete and minimal media. The isolates that had survived 10 days of starvation were examined in terms of plasmid and chromosomal DNA. Results have showed significant chromosome changes and plasmid losing in cells grown in complete media but not when they were grown in the minimal medium.

Adenylation domain of nonribosomal peptide synthetase - the role of conserved motifs and protein-protein interactions

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Nonribosomal peptide synthetases (NRPS) are modular proteins that catalyze the synthesis of small peptides with antibiotic, immunosuppressant, and anticancer activities, as well as siderophores. NRPS usually contain one module for each amino acid incorporated into the final peptide. Each module consists of several catalytic domains that catalyze the activation of specific amino acids (adenylation (A) domain), covalent thioester binding (peptidyl-carrier-protein (PCP) domain), formation of peptide bond (condensation (C) domain), and optionally, various substrate modifications. A domain catalyzes the two-step reaction of ATP-driven activation of amino acid, followed by its transfer to PCP domain. Sequence alignments of A domains allowed identification of 10 'core motifs'. Most of them were assigned particular functions, thanks to crystal structures and mutagenesis. Unequivocal function for several conserved motifs has not been established so far. Recently, a significant progress in mapping protein interactions between individual NRPS domains has been achieved. However, regions important for A and PCP domain interaction were not identified. In our previous work we were studying the fidelity of substrate selection by A domain, using tyrocidine synthetase from *B. brevis* as a model system. Our present work is focused on investigating the role of conserved sequence motifs, especially those that are shown to adopt strikingly different conformations during the two-step catalytic reaction. We are also interested in examining A domain for putative protein-protein interaction surfaces. We hope that the results obtained in these studies will facilitate the rational design of NRPSs as a means of producing peptides with novel biological activities.

LOH of Rb1 gene in sporadic colon cancer

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Colon cancer arises from the accumulation of mutations during progression from normal colon epithelium to adenoma and carcinoma. In addition to the activation of oncogenes, there is an inactivation of tumor suppressor genes via frequent allelic deletions. One of the tumour suppressor genes that are considered to be involved in the development of colon cancer is Rb1. The aim of this study was to evaluate the loss of heterozygosity at the polymorphic locus inside Rb1 gene in sporadic colon cancer. The investigated locus was a CTTT(T) repeat in intron 20. Normal tissue samples from 150 sporadic colon cancer patients were genotyped in order to determine the frequency of heterozygosity at this locus. Corresponding tumour tissue samples were then analyzed to determine the LOH status. We also compared the results between genders to find if the distributions were gender-related.

The frequency of heterozygosity was 84% at the microsatellite locus in intron 20 and LOH was found in 9% of tumour samples. No significant differences were found between genders.

Lipopolysaccharide induces increased bone resorption by stimulating homing of osteoclast progenitors to the periosteal bone surface

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Background/aims: Lipopolysaccharide (LPS) from gram-negative bacteria causes chronic inflammation and subsequent bone loss, and is involved in the pathogenesis of several bacterially induced bone diseases. We investigated the effects of LPS on bone metabolism and osteoclast differentiation from hematopoietic cells.

Methods: C57BL/6 mice were injected during 4 weeks in a dose of 10 µg LPS/g body weight and sacrificed at different time-points. Cells from different tissue sources (bone marrow, homogenized bone shafts, spleen and peripheral blood) were cultured with RANKL (40 ng/mL) and M-CSF (15 ng/mL) to stimulate osteoclast differentiation. Osteoclasts (OCL) were identified as TRAP positive multinucleated cells with three or more nuclei per cell. Femoral sections (5 µm) were stained with Goldner-trichrome and TRAP staining. Microtomography (µCT) was performed by SkyScan1172 high resolution micro-CT. OCL progenitors were characterized by flow cytometry as a population negative for lymphoid markers (B220, CD3, NK1.1) and positive for CD115 and/or CD117, within both CD11b negative/low and CD11b positive populations. Gene expression analysis of OCL differentiation genes was performed by qPCR.

Results: Three weeks after LPS stimulation, the number of OCL differentiated from cells extracted from bone shafts (504.8 ± 74.28) was higher compared with control mice (383.3 ± 30.48 ; $p=0.0025$). This was in correlation to the decrease in bone volume and trabecular thickness detected by µCT. Femoral sections showed that LPS treatment altered bone metabolism in vivo by inducing increased osteoresorption in bone cortex starting from the periosteal bone surface. This was confirmed by gene expression analysis of bone shafts, showing increased expression of OCL differentiation genes including RANK and cFms. Flow cytometry indicated that enhanced bone resorption starting at the periosteal surface may be caused by homing of peripheral OCL progenitors, since we found approx. 2-3 fold increase of OCL progenitor cell populations in peripheral blood and spleen 10 days after LPS treatment. This was confirmed by increase in number of differentiated OCL from both blood (181 ± 32.16) and spleen (299.25 ± 61.37) compared to controls (blood 43.8 ± 25.7 ; spleen 189 ± 54.88).

Conclusion: LPS administration stimulates homing of OCL progenitors to periosteal bone surface and supports osteoclast differentiation. Our further aim is to identify factors induced by LPS that mediate this osteoclastogenic effect and cause enhanced bone resorption.

The aminoglycoside resistance methyltransferase Sgm impedes RsmF methylation at an adjacent rRNA nucleotide in the ribosomal A site

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Sgm methyltransferase is a member of the Arm family of enzymes that confer resistance to aminoglycoside antibiotics by modifying the ribosomal RNA within the binding site of the aminoglycosides, the A site of the ribosome. The Sgm enzyme was isolated from sisomicin producing actinomycete *Micromonospora zionensis*, where it protects the cell from the toxic effect of the antibiotic. Recently, some new members of this family have emerged in clinical practice, posing a serious threat to the successful treatment of various bacterial infections with the aminoglycosides. Therefore, there is a great need for the functional characterisation of these enzymes.

In all kingdoms of life, a series of covalent modifications in ribosomal RNA is needed for the proper assembly of the ribosome. In bacteria, the most common type of rRNA modification is the methylation of the base, followed by the methylation at the 2'-O-position of the ribose and the conversion of uridine to pseudouridine. Each indigenous rRNA methylation in bacteria is carried out by its own specific housekeeping methyltransferase. It is therefore interesting how an extra methylation conferring antibiotic resistance can be exerted within a heavily modified rRNA region.

In this work we show that the Sgm methyltransferase confers resistance to 4,6-disubstituted deoxystreptamine aminoglycosides by introducing the 16S rRNA modification m7G1405 within the ribosomal A site. In the vicinity of the nucleotide G1405 there are several nucleotides methylated by housekeeping methyltransferases, such as m4Cm1402 and m5C1407. We also show that the modification at m5C1407 by the methyltransferase RsmF is impeded as Sgm gains access to its adjacent G1405 target on the 30S ribosomal subunit.

Our data indicate that methyltransferases that cause antibiotic resistance due to the methylation of the functionally important region of rRNA could affect the indigenous rRNA methylation caused by housekeeping methyltransferases and thus play an important role in determining resistance levels.

Plant Hormones Auxins Disturbe Cell Cycle of Human Tumor Cells

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Phytohormones auxins are the key regulators of plant growth and development. Recently, it has been shown that they regulate transcription of plant genes by targeting transcriptional repressor IAA/AUX to degradation. While there are many reports describing their potential to modulate human cell functions, the majority is based on auxin action following enzymatic activation. The here-presented study is focused on auxins alone and their antiproliferative potential, with emphasis on the modulation of the cell cycle. Therefore, the tumor growth inhibitory effect and the cell cycle perturbations of natural (IAA, IBA) and synthetic (NAA, 2,4-D) auxins were analyzed. All derivatives showed cytostatic effect on selected human tumor cell lines. The cell cycle analysis revealed that IAA and 2,4-D induce strong G1 arrest, along with drastic decrease of S phase-cells percentage in MCF-7 cell line. The observed effect points that auxins may have a novel, unexploited antitumor potential, and should be further investigated in this regard.

Bee venom and melittin display cytotoxicity and alters morphological appearance of different types of tumor and non-tumor cell lines

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The use of venoms from various organisms has a long history in traditional and alternative medicine. In the past few years a number of studies regarding beneficial role of bee venom were published indicating its radioprotective, antimutagenic, anti-inflammatory, antinociceptive and anticancer activities. Due to the wide range of effects induced by both, bee venom and its major constituent melittin on different types of human cells, these natural products became a very interesting model in anticancer research. The first step in such investigation is to determine their cytotoxicity on various tumor and non-tumor cell lines, and to examine the morphological alterations and the type of the cell death that they induce. Accordingly, this was the aim of this study. Using nine different cultured human cell lines (human laryngeal HEp-2 and cervical carcinoma HeLa cells, breast adenocarcinoma MCF-7 cells, colon adenocarcinoma SW620 cells and glioblastoma A1235, as well as non-tumor human embryonic kidney HEK-293 cells and Hef fibroblasts), we explored the cytotoxic effect of bee venom and melittin. As the major obstacle for successful treatment of tumor patients treated with the standard chemotherapy is the development of drug resistance in tumor cells during the course of chemotherapy, we also included in this study two cell lines resistant to the standard anticancer drugs (CK2 and HeLa CK cells). In that manner, bee venom was tested in concentrations ranging from 0.4 µg/ml to 200 µg/ml, and melittin in concentrations from 0.1 µg/ml to 50 µg/ml. Cytotoxicity of whole bee venom, and melittin was evaluated using spectrophotometric MTT test whereas the morphology of treated cells was determined by light and fluorescent microscopy. Our results show that bee venom and melittin have strong cytotoxic potential towards different human cultured tumor cells, and that their effects are dose and cell type dependent. Melittin displayed even greater cytotoxicity to all types of cells tested. In addition, tumor cells were more sensitive to both, bee venom and melittin, as compared to the non-tumor cells. Depending on the tissue of origin, drug-resistant cells could be more sensitive to melittin than parental cells. Light microscopy showed that both, bee venom and melittin caused significant morphological changes: rounded and granulated cells, shrinkage and eventual detachment from the culture plates. These alterations were induced rapidly following the addition of bee venom or melittin. Staining with fluorescent dye ethidium bromide suggest the damage of the cell membrane as the cause of cell death. Bee venom and melittin induced what appeared to be necrotic type of cell death in cells treated with high concentrations of both compounds. In conclusion, our results indicate that bee venom and melittin have strong cytotoxic potential towards different human cultured tumor cells, and their effects were dose and cell type dependent. In addition, tumor cells were more sensitive to both, bee venom and melittin, as compared to the non-tumor cells. Therefore, our data in conjunction with other accumulating evidence on anti-proliferative and pro-cell death activity of bee venom and melittin indicate their possible use in the development of antitumor drugs.

Aminoacyl-tRNA synthetase complex in methanogenic archaea

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Seryl-tRNA synthetases are essential enzymes for protein biosynthesis. Moreover, an elaborate network of protein-protein interactions of aminoacyl-tRNA synthetases is required for efficient translation in all domains of life. Several aminoacyl-tRNA synthetases (aaRS) are located in multi-synthetase complexes (MSC) in mammals. Identification of the network that connects possible regulatory non-synthetase proteins to synthetases or synthetases to each other and to the cellular processes they affect is a critical need. Archaeal SerRSs diverge into two major and disparate types of enzymes (bacterial and methanogenic type). We revealed protein partners of methanogenic type seryl-tRNA synthetase (SerRS) in *Methanothermobacter thermautotrophicus* using yeast two-hybrid screen that facilitates construction of protein-protein linkage maps. *M. thermautotrophicus* arginyl-tRNA synthetase (ArgRS) was found interacting with SerRS as a result of the screen. We have isolated and kinetically examined *M. thermautotrophicus* SerRS to gain biochemical insight into the complex. Further, dissociation constant of SerRS:ArgRS complex was determined by surface plasmon resonance. Interestingly, the biological significance of interaction SerRS with ArgRS is improvement of SerRS enzymatic activity two- to five-fold. Before, SerRS has never been found interacting with another synthetase or within the MSC. This type of structural organization is not restricted to eukaryotic species because archaeal requirements for aaRS associations are becoming more evident, as these proteins can function together in many important biological contexts such as translation and substrate channeling.

Bisphenanthridinium-nucleobase conjugates – molecular modelling and spectroscopic approach

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The subject of this work is study on nucleobase conjugates of bisphenanthridinium and their complexes with nucleotides. It shows how molecular modelling, particularly the long time molecular dynamics simulations (MD) can be used to rationalize the experimental results on molecules and their complexes. Accordingly, molecular modelling results in combination with the spectroscopic measurements for a series of nucleobase conjugates of bisphenanthridinium as well as their non-covalent complexes with UMP and AMP will be presented. AMBER ff031 and GAFF2 force fields were used to parametrize molecules and Molecular Dynamics (MD) simulations of solvated molecules and their complexes were accomplished with the program AMBER92. During the MD simulations initial folded conformations of some molecules relaxed; however majority of molecules remained in their folded, more or less stacked conformation with no water molecules accommodated within the two phenanthridinium rings. The adenine derivative of bisphenanthridin nucleobase conjugates revealed highly selective affinity toward complementary nucleotide. The initial structure of its complex with UMP was built in a way to enable adenine and uracil from UMP to form the Watson-Crick type of H-bonds. During the MD simulation the conformation of the complex changed and stabilized in a conformation with adenine in the stacking interaction with one phenanthridinium ring, and uracil with the other. In this interaction the hydrophobic pocket made by two phenanthridinium rings and the alkyl linker is very functional since there is no water molecule within it to compete with uracil from UMP for the hydrogen bond.

The obtained results performed for its complexes with AMP and UMP are in agreement with the experimentally determined spectroscopic results and the measured binding affinities.

IGF2 promoter usage and expression of IGF2AS are regulated by IGF2-Bi methylation, the presence CTCF and poly(ADP-ribosylation)

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The maintenance of IGF2/H19 imprinting depends on the methylation of H19 ICR, the presence of CTCF and ADP-ribosylation. The transcription of IGF2 is regulated by five promoters in a tissue- and age-specific manner. The imprinted IGF2 has an antisense transcript originating from the promoter located within a CpG island near IGF2 promoters P2-P4. The IGF2-Bi fragment in this region has insulator properties and contains methylation-sensitive CTCF-binding sites. Our aim was to determine the influence of DNA methylation and ADP-ribosylation on the regulation of IGF2 promoter usage and IGF2AS transcription.

Human tumor cell lines HT-29 and Cal-27 were chosen for this study based on different global DNA methylation (HT-29 being 2.2 x more methylated than Cal-27) and the ADP-ribosylating capacity (lower in HT-29). They were treated with PARP-1 inhibitor, 3-aminobenzamide (3-AB), to inhibit poly(ADP-ribosylation) and promote DNA methylation, or 5-azacytidine (5-azaC) to induce DNA demethylation. DNA methylation of DMR0 and IGF2AS promoter was analyzed by COBRA. ADP-ribosylating capacity was estimated based on the elevation of NAD level in the cells after 3-AB treatment. Expression of IGF2AS was analyzed by qPCR. The promoter specific transcripts of IGF2 were analyzed by RT-PCR.

Both untreated cell lines had hypomethylated IGF2AS promoter to which CTCF could bind, but IGF2AS was expressed only in HT-29 cells with low ADP-ribosylating capacity. The IGF2 promoter usage differed: in HT-29 cells IGF2 transcription originated from promoters P1, P0, P3 and P4 but not from P2, although P2 transcripts including alternative exon 4b (P2/4b) were present. In Cal27 cells, only promoters P3 and P4 were active. The DMR0 was hemimethylated in HT-29 and hypomethylated in Cal-27 cell line.

Treatment with 5-azaC, which causes DNA demethylation and inhibits poly(ADP-ribosylation), downregulated IGF2AS expression in HT-29 cells by 60% most likely due to the inability of AP-1 to bind IGF2AS promoter in the complete lack of ADP-ribosylation. The same treatment had no effect on IGF2AS expression in Cal-27 cells but caused upregulation of IGF2 P2 and the appearance of P2/4b transcripts, likely due to the inhibitory effect of 5-azaC on ADP-ribosylation. The P1 and P0 promoters remained silent in Cal27 cells. No changes in methylation of IGF2AS promoter and DMR0 were observed in either cell line.

Upon 3-AB treatment, the expression of IGF2AS was downregulated in HT-29 cells by 30% concomitant with emergence of very low level IGF2 P2 transcripts. Methylation status of IGF2AS promoter and transcription of IGF2 from other promoters has not changed, while DMR0 methylation

has increased. The expression of IGF2AS was still undetectable in Cal27 cells upon 3-AB treatment. This treatment resulted in appearance of IGF2 P2 and P2/4b transcripts. No changes in methylation of IGF2AS promoter or DMR0 were detected.

Our results suggest that in tested cell lines: 1) IGF2AS and IGF2 P2 transcription are mutually exclusive; 2) binding of CTCF to hypomethylated IGF2-Bi element prevents transcription from P1, P0 and P2/4b only in poly(ADP-ribosylation)-competent cells; and 3) IGF2 transcription originates from P1 and P0 only in IGF2AS-expressing cells, regardless of DMR0 methylation.

Sialic Acid Synthesis in *Aliivibrio salmonicida*

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Sialic acids are nine carbon sugars found associated with both bacterial and eukaryal cells. They are vastly important for cell-cell communication, pathogen interaction and immune recognition. Sugar modifying enzymes can be utilized in many different areas in molecular biology and medicine. The combination of chemical and enzymatic synthesis will bring forward new saccharides which can have useful properties. The genome of the fish pathogen *Aliivibrio salmonicida* LFI1238 reveals that it possesses the ability to synthesize the two sialic acids Neuraminic Acid and Legionaminic acid, the latter of which is found as the terminal O-antigen sugar. Neuraminic acid has not previously been reported in *A. salmonicida*. Homologous genes are, however, involved in synthesis of Neuraminic acid found in the *E. coli* capsule. We have recombinantly expressed nine of the proteins belonging to the pathways leading to these sugars. Since *A. salmonicida* is a psychrophile, it might also produce cold adapted versions of the enzymes, which renders them highly interesting as targets for commercial exploitation.

Effects of carbonylation on the stability of proteins

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Proteins frequently become irreversibly modified by carbonylation, a process of introducing the carbonyl group in a reaction with reactive oxygen species (ROS) such as superoxide, peroxide or ozone. The main targets for carbonylation in proteins are amino acid side chains of lysine, arginine and proline. Products of carbonylation are aminoadipic semialdehyde from lysine (ASA) and glutamic semialdehyde (GSA) from arginine and proline. Importantly, carbonylated proteins are marked for proteolysis by the proteasome, but can escape degradation and form aggregates that can be cytotoxic. Carbonylation increases with the age of cell and it is associated with ageing and age related disorders such as Alzheimer's disease, Parkinson's disease and cancer. We have used molecular dynamics approach to study the stability of carbonylated villin headpiece protein. Simulations were run after mutations of arginine, proline and lysine into GSA and ASA had been performed. In addition, we have used thermodynamic integration on lysine, arginine, proline, ASA and GSA residues in order to estimate their solvation free energy (related to relative hydrophobicity and hydrophilicity). Our results suggest that carbonylation markedly decreases the overall stability of proteins, and that one potential reason for that may be a disruption of the balance between hydrophilic and hydrophobic regions in the protein.

Expression of Nanog, PDX-1, ErbB-3 and Insulin after transplantation of bone marrow stem cells in diabetic mice

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The islets of Langerhans are little islands of cells, discovered by Paul Langerhans. The islets of Langerhans contain three major types of cells: a) alpha cells which produce glucagon; b) beta cells which produce Insulin; and d) delta cells which secrete somatostatin.

Diabetes mellitus, commonly known as diabetes, is one of the world's oldest known diseases. Diabetes is a chronic disorder characterized by high blood sugar levels and abnormal metabolism of carbohydrate, protein and fat. Diabetes is caused by a deficiency of the hormone insulin (Type 1 diabetes mellitus) or the body's inability to use insulin (Type II diabetes mellitus). Insulin is produced in by beta cells located in the islets of Langerhans of the pancreas.

High blood glucose levels, during long periods of time, causes proteins glycation and inducing secondary complications: a) retinopathy which may lead to blindness; b) neuropathy (nerve degeneration) which may lead to gangrene, and c) nephropathy which may lead to kidney malfunctions.

In our experiments diabetes was induced in CBA/HZgr mice by treatment with alloxan (75 mg/kg/bw). The Alloxan is a chemical compound that causes irreversible damage to pancreatic b-cells and induces persistent hyperglycemia. To regenerate damage islets we transplanted in alloxan-induced diabetic mice syngeneic bone marrow stem cells. The diabetic mice were received the 8×10^4 bone marrow stem cells. The pancreas and spleen of this study are analyzed by expression of Nanog, PDX-1, ErbB-3 and Insulin by immune histochemistry.

The blood sugar of hyperglycemic animals treated with bone marrow stem cells, 24h after administration of alloxan, was recovered within 14 days and some of them returned to the normal value (in 7 of 10 mice). Moreover, after 14 days of transplantation we see regeneration of the pancreatic beta-cell. For conclusion, the histological survey of these bone marrow stem cells treated diabetic animals revealed injure pancreatic islets that were filled with new beta-cells.

Retargeting Adenovirus Type 5 to Integrin $\alpha 4\beta 1$

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Gene therapy by means of recombinant adenovirus type 5 (Ad5) vectors could become a unique approach in treating certain types of cancer, among which acute myeloid leukemia (AML). The majority of leukemic cells lack CAR expression on their cell surface, resulting in resistance to Ad5 infection. However, AML cells have increased expression of $\alpha 4\beta 1$ (VLA-4) integrin on their cell surface for which targeting ligand CPLDIDFYC has been recently isolated. The aim of this research is to construct a replication defective adenoviral vector by incorporating $\alpha 4\beta 1$ integrin-targeting ligand into the HI-loop of the adenovirus particle fiber protein, in order to obtain an Ad5 vector capable of efficiently infecting AML cells. Construction of Ad5VLA4 was performed by inserting a duplex of oligonucleotides coding for CPLDIDFYC into the portion of the Ad5 sequence encoding the HI-loop, and manipulation of the full length Ad5 genome as a stable plasmid in *E. coli*, using the bacterial homologous recombination machinery. All constructions were further confirmed by partial sequencing of the fiber gene. Both types of viruses, Ad5VLA4 and the control virus Ad5wt lacking targeting sequence, were multiplied in human embryonic kidney cell line-293 cell culture and purified by bending in CsCl gradients. The infectivity indices on 293 cells (the ratio of physical particles to infectious particles) of CsCl purified virus preparation were similar showing that incorporation of ligands in HI-loop did not change virus infectivity. On the rhabdomyosarcoma (RD) cell line, which express $\alpha 4\beta 1$ integrin, Ad5VLA4 exhibited increased transduction efficacy in comparison to Ad5wt. Therefore, Ad5VLA4 could be suitable vector for gene therapy of tumors expressing $\alpha 4\beta 1$ integrin. In continuation of this work we want to test whether or not Ad5VLA4, in comparison with wild type Ad5, would also show better transduction of human JURKAT cell line expressing VLA-4. If successful, we plan to construct another adenovirus vector expressing the herpes simplex thymidine kinase (HSV-TK) suicide gene that would make tumor cells sensitive to nucleoside analogs such as ganciclovir (GCV). This vector could be used in treating acute myeloid leukemia in vivo.

Analzsis of expression of Stam2 gene using a gene trap derived transgenic mouse model

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STAM2 (Signal transducing adaptor molecule 2) is a phosphotyrosine protein taking part in an endosome associated complex containing also HRS and EPS15 proteins. This complex has been suggested to be involved in sorting of mono-ubiquitinated endosomal cargo toward degradation in the lysosome. As STAM2 is phosphorylated upon binding diverse growth factors and cytokines to the cell membrane, it might also play a regulatory role in cell signalling, being at the intercross of signalling pathways and membrane transport in the cell.

As a consequence of gene trap modification, lacZ gene is in frame with Stam2, therefore expression pattern of Stam2 can be assessed by simple histochemical detection of beta-galactosidase activity via its substrate X-gal. This was used to determine Stam2 expression pattern in the mice carrying gene trap insertion. The advantage of this approach is that the expression is detected on protein level.

To verify the above results in situ RNA hybridization using radioactive (S35) labelled probe was made.

Stam2 is expressed in the central nervous system in the regions containing numerous neuron cell bodies, i.e. mainly in the cortex and hippocampus. The expression of Stam2 is also in the peripheral nervous system under study(i.e. in the trigeminal ganglion, in dorsal root ganglia and in the enteric nerve networks).

In order to further clarify which cell types are expressing Stam2, three immunohistochemical markers were checked for colocalisation with beta-galactosidase: PGP as a general neuronal marker, GFAP as a glial cell marker, and c-kit as marker for interstitial cells of Cajal. No GFAP-immunoreactive glial cells were observed to stain for beta-galactosidase. Some co-labeling for beta-galactosidase was noticed in c-kit immunoreactive interstitial cells of Cajal within the myenteric plexus. The majority of the beta-galactosidase positive cells, however, contained for PGP, indicating that the most of these Stam2-expressing cells were neuronal in origin.

As STAM2 together with HRS (hepatocyte growth factor regulated tyrosine kinase substrate) forms the ESCRT-0 complex implicated in sorting of ubiquitinated receptors toward late endosomes/multivesicular bodies and subsequent degradation in the lysosome, the observed expression suggests the importance of Stam2 in the regulation of growth factor and cytokine signalling in the nervous system.

Cholesterol accumulation upon NPC1 dysfunction alters endosomal compartmentalization of β -amyloid precursor protein

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Dysfunction of NPC1 protein causes Niemann Pick type C disease (NPC), a lysosomal storage disorder that is characterized by accumulation of cholesterol and glycosphingolipids in endosomal/lysosomal compartments. It has been recently demonstrated that cholesterol accumulation upon loss of NPC1 function leads to altered processing of the β -amyloid precursor protein (APP) causing increased β -amyloid peptide formation ($A\beta$), the causative factor of Alzheimer's disease (AD). The goal of this work was to investigate whether altered APP processing in NPC disease could be due to cholesterol-mediated trafficking defect of APP. To test this we analyzed cell surface expression, subcellular distribution and endosome compartmentalization of endogenous APP and presenilin 1 (PS1) in CHOwt and CHO NPC1-null cells. Biotinylation assay showed a marked decrease of APP at the cell surface in NPC vs. wt cells, suggesting that NPC1 loss may cause a shift in APP distribution within subcellular compartments. Indeed, subcellular and endosome fractionation confirmed that in NPC cells there is more APP and PS1 within early endosomal compartments compared to wt cells. Our results show that cholesterol accumulation upon loss of NPC1 function alters trafficking of APP/PS1 towards endosome compartments, supporting that increased localization of APP/PS1 within endocytic pathway leads to increased $A\beta$.

Intratumoral heterogeneity of E-cadherin and beta-catenin in laryngeal squamous cell carcinoma

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E-cadherin and beta-catenin are membrane-associated proteins involved in cell-cell adhesion. Their dysfunction leads to adhesiveness loss, enabling neoplastic cells to invade surrounding tissue and metastasize. Carcinogenesis is a series of genetic defects leading to aberrant cell proliferation and eventually to intratumoral heterogeneity. Zonal intratumoral heterogeneity possibly plays an important role in tumor biology. In our work the heterogeneity of E-cadherin and beta-catenin expression was investigated as well as their correlation with tumor differentiation.

The study included 47 patients with laryngeal squamous cell carcinoma operated at the ENT clinic KBC Zagreb, in the period 2003-2005. Tissue samples were fixed in 4% buffered formalin and embedded in paraffin. Three um sections were cut and stained with HE and immunohistochemically using anti E-cadherin and beta-catenin antibodies (DAKO). The reaction was assessed using a scoring system (0-3), estimating membrane and cytoplasmatic immunopositivity at three separate locations- the transformation zone, the centre and at the invasive segment of the tumor.

A significant difference of expression between the tumor regions was found for both membrane and cytoplasmatic beta-catenin, and membrane E-cadherin. The invasive segment showed the poorest membrane reaction. No correlation was found between the expression of these molecules and the differentiation of the tumor.

Variability, heritability and environmental determinants of human plasma N-glycome

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Plasma glycans were analyzed in 1008 individuals to evaluate variability and heritability, as well as the main environmental determinants that affect glycan structures. By combining HPLC analysis of fluorescently labeled glycans with sialidase digestion, glycans were separated into 33 chromatographic peaks and quantified. A high level of variability was observed with the median ratio of minimal to maximal values of 6.17 and significant age- and gender-specific differences. Heritability estimates for individual glycans varied widely, ranging from very low to very high. Glycome-wide environmental determinants were also detected with statistically significant effects of different variables including diet, smoking and cholesterol levels.

Functional analysis of Human TLR9 Gene Variants

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Toll-like receptors (TLRs) are thought to be the link between the mammalian innate and adaptive immune responses. Cells expressing TLRs sense the presence of conserved molecular products derived from different microorganisms, including bacteria, viruses and fungi. TLR9 molecules bind microbial and synthetic unmethylated DNA rich in CpG motifs. Stimulation with specific ligands initiates a signaling cascade that leads to the activation of the NF- κ B transcription factor. The aim of this study was to detect and functionally characterize the extent of TLR9 gene variation in Croatian population. Complete coding region of TLR9 gene was sequenced from blood-cell-derived genomic DNA of 200 individuals. The analysis revealed eleven gene variants; four of them were missense, resulting in change of amino acid sequence: 175delG (111X), C200 \rightarrow A (F667L), G2588 \rightarrow A (R863Q) and C2674 \rightarrow T (R892W). Functional analysis by transfection assay using luciferase as the reporter gene revealed inhibition of signaling, as ascertained by measuring NF- κ B activity in HEK293 cells; 111X and R892W completely lacked the ability to upregulate the receptor gene activity, while F667L and R863Q counterparts were hypo-reactive. All these variants showed a dominant negative effect in double-allele cotransfections with the wild type TLR9.

Determination of root-mean-square pairwise RMSD for an ensemble of structures from experimental B-factors

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Root-mean-square deviation (RMSD) is a measure used to give information on the global structure of macromolecules. For example, pairwise RMSD is used to assess similarity of the lowest energy NMR structures or for clustering large ensembles of structures. On the other hand, to obtain information on the local structure of a macromolecule and its dynamics, root-mean-square fluctuations (RMSF) are often used. RMSF can be calculated from MD simulations, but also from experimental X-ray B-factors (Debye-Waller or temperature factors). Since RMSD and RMSF report on different features, it is interesting to ask what the relationship between them is. First, we provide a mathematical derivation showing that, given a set of conservative assumptions, the $\langle \text{RMSD}^2 \rangle^{1/2}$ is directly related to the $\langle \text{RMSF}^2 \rangle^{1/2}$ and, consequently, experimental B-factors. Second, we demonstrate this on structures taken from distributed-computing MD simulations of the native and unfolded state of villin headpiece domain. Both our analytical and computational results suggest a direct proportionality between two measures: $\langle \text{RMSD}^2 \rangle^{1/2} = [2S/(S-1)]^{1/2} \langle \text{RMSF}^2 \rangle^{1/2}$, where S is the number of compared structures. Furthermore, if $\langle \text{RMSD}^2 \rangle^{1/2}$ is defined as a generalized radius of gyration in the space of 3D structures and using RMSD as a measure of distance, the following identity holds: $\langle \text{RMSD}^2 \rangle^{1/2} = \langle \text{RMSF}^2 \rangle^{1/2}$. Our results provide a basis for determining the level of structural diversity of molecular ensembles, as captured by $\langle \text{RMSD}^2 \rangle^{1/2}$, directly from experiment.

**Determination of genetic variability in Croatian wild boar (*Sus scrofa* L.)
population using DNA microsatellite markers**

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The existence of high diversity between animal populations and species, negative human influence through overexploitation, as well as changes of natural habitats, both ensured the fact that animal entered into the focus of many molecular biological investigations. As a consequence of the 1991-1995 Homeland War there was a genomic hybridization between domestic pig and wild boar. To utilize a distinction between these two genomes a novel pig microsatellite panel was designed for determination of genetic variability in Croatian wild boar population. This study included 269 samples from 16 different wild boar populations throughout Croatia. All samples were genotyped for 14 microsatellite loci chosen according to their reproducibility, high polymorphism and absence of null alleles. Microsatellite loci are grouped into three Polymerase chain reaction multiplexes (2-plex, 5-plex and 7-plex) depending on required annealing temperature, allele size range and primer fluorescent label. All the microsatellite loci in these three multiplexes showed expected heterozygosity and diverse allele size ranging from 90 to 248 base pairs. Results show that they could be used for identification and determination of genetic linkage between two organisms with high probability.

The Efficiency of Gene Replacement in Yeast is Allele-Specific

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Introduction of linear, non replicative DNA fragments is widely used for the replacement of homologous sequences in the yeast genome. This procedure is very useful for the construction of different yeast strains but is also studied as a general model for targeted genetic manipulations by the "ends-out" recombination. It is generally assumed that, in contrast to higher eukaryotes, transformation of the yeast *Saccharomyces cerevisiae* with non-replicative DNA occurs almost exclusively by homologous recombination. However, we found out that the ends-out recombination may result in different genetic alterations in the yeast genome and we performed a systematic study of genetic events that may arise as side-effects of gene replacement. In our experimental systems, different URA3 alleles were present on the transforming DNA and in the yeast genome while the spectra of transformation events were determined by Southern blot hybridization. Depending on the experimental system targeted gene replacement ranged from only 60 % to 98 %. Molecular analysis of transformants revealed versatile aberrant recombination events that included illegitimate integration of the transforming DNA, gene or whole chromosome duplications and chromosomal rearrangements. We show that the fidelity of gene targeting in yeast is mainly influenced by the nature of alleles involved in recombination. Additionally, our results suggest that the ends-out recombination involving endogenous DNA fragments may have an important role in the evolution of the yeast genome.

MM calculations of unit cell packings of aquabis(L-valinato)copper(II) in simulated crystal lattice

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Bis(amino acidato)copper(II) complexes are biologically important compounds. They take part in transporting copper to copper enzymes (such as superoxide dismutase, cytochrome c oxidase and nitrous-oxide reductase) and electron-transfer copper proteins (e.g., plastocyanin, azurin). The X-ray crystal structures are the most common source of experimental information on the structural properties of this class of compounds.

Bis(L-valinato)copper(II), Cu(L-Val)_2 , crystallised from aqueous solution as a *cis*-isomer with one water molecule in the asymmetric unit (space group C2), as determined by the X-ray diffraction measurements. The coordination geometry of the copper(II) is a distorted pyramid, with a water oxygen atom at the pyramid apex. The molecules are bonded together via intermolecular $\text{N-H}\cdots\text{O}_{\text{carbonyl}}$, $\text{N-H}\cdots\text{O}_{\text{carboxyl}}$ and $\text{O}_{\text{water}}\text{-H}\cdots\text{O}_{\text{carbonyl}}$ hydrogen bonds.

Molecular mechanics force field FFWa-SPCE [1], developed for studying the properties of bis(amino acidato)copper(II) complexes with either *cis*- or *trans*-N2O2 copper(II) coordination geometry in the solid state and *in vacuo* by MM calculations, and in aqueous solution by MD simulations, was used for conformational analysis of the title compound.

Each chelate ring of Cu(L-Val)_2 can have 6 conformations, with C^β in 3 axial and 3 equatorial positions, and therefore the molecule can have 21 *trans* and 21 *cis* conformations. Conformational analysis *in vacuo*, without the influence of the intermolecular interactions, showed that the *trans* conformers were more stable than the *cis* ones (by $\approx 102 \text{ kJ mol}^{-1}$). To account the crystal lattice effects, the experimental molecule orientation, unit cell lengths and angles, as well as the C2 space group symmetry operations were taken as the starting input data for the geometry optimisation of all possible conformers. During the energy minimisation of a crystal all degrees of freedom were allowed to vary, except the α and γ unit cell angles were kept fixed to 90° . The calculations of the unit cell packings and intermolecular interactions for the series of conformers suggest the reasons why experimentally obtained conformer occurs in the real crystal structure.

SAHH deficiency - Molecular mechanisms of a new genetic disorder

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The human disease, S-adenosylhomocysteine hydrolase (SAHH) deficiency was discovered 2004 in Croatia. SAHH catalyses the hydrolysis of AdoHcy, the product inhibitor of AdoMet-dependent methyltransferases. Therefore, understanding of SAHH deficiency is crucial for methylome research. AdoMet-dependent methylation affects many aspects of cellular function including epigenetics, protein processing, lipid biosynthesis and signalling. Furthermore, elevated levels of homocysteine inhibit AdoMet-dependent methylation, thereby mimicking SAHH dysfunction. Our aim is understanding of the impact of SAHH deficiency on cellular metabolism as well as methylation-mediated aspects of homocysteine-associated disorders, including histone methylation.

Enzymes that belong to different protein families can methylate both lysine and arginine residues from histones H3 and H4. While arginines can be either mono- or dimethylated, lysines can be mono-, di- or trimethylated. During this part of the project we will mainly focus on histone lysine methylation because – in contrast to histone arginine methylation which is mainly linked to transcriptional activation – this modification could be associated to either activation or repression of the transcription, depending on the site and status (mono-, di-, or tri-) of the methylation of histone lysine residues. Moreover, in contrast to other histone modifying enzymes, histone lysine methyltransferases are enzymes devoted to methylation of highly specific lysine residues. In addition, Drosophila genetics led to the emergence of the concept that histone lysine methylation is involved in the maintenance of different types of active and repressed chromatin and therefore plays a role in propagating chromatin states as an epigenetic memory. Particular objectives of this part of the project are;

- Generation of RNAi model cell lines for SAHH deficiency
- Description of chromatin structure in SAHH deficiency
- Defining the status of histone lysine methylation in RNAi cell lines
- Finding potential protein biomarker for SAHH deficiency
- Contributing to protein methylation interactome-protein methylome

We believe that our approach will lead to the unraveling of the mechanisms underlying the SAHH genetic disorder and contribute to the better understanding of processes in the eukaryotic methylome.

The Role of Galectin-3 in Immune Cells Activation

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Galectin-3, a β -galactoside binding lectin exerts important roles in many (patho)physiological processes (adhesion, proliferation, differentiation, apoptosis, inflammation, neoplastic transformation, spreading metastases). Being one of the key lectins of innate and acquired immunity, galectin-3 is considered a powerful pro-inflammatory signal. It triggers/promotes respiratory burst in monocytes, acts as a monocyte/macrophage chemoattractant and promotes survival of inflammatory cells through its anti-apoptotic activity.

The aim of this study was to ascertain the level of galectin-3 expression and explore its role in (patho)physiological processes of monocytes and lymphocytes. Flow cytometry was used to determine the level of membrane and intracellular galectin-3 expression on a model THP-1 monocytic cell-line. We used lipopolysaccharide (LPS) activated and phorbol 12-myristate-13-acetate (PMA) differentiated, THP-1 cells cultured in RPMI 1640, supplemented with FCS and antibiotics. Galectin-3 was detected using anti-human galectin-3 primary, and labeled secondary antibodies. Dead cells were excluded by 7AAD. Macrophage-like morphology was confirmed by an elevated CD14 stain and surface adhesion. LPS-activated THP-1 cells have markedly up-regulated expression of intracellular galectin-3, while the surface level remains largely unchanged. Differentiation of monocytes to macrophages is associated with an increase of surface galectin-3 in respect to control cells.

Using cytokine capture beads, we also studied the effects of recombinant galectin-3 on inflammatory cytokine secretion by human PBMC's from healthy volunteers. It was shown that exogenous galectin-3 influences cytokine secretion in human monocyte derived macrophages and lymphocytes.

Given the importance of galectin-3 in monocyte and lymphocyte physiology, further experiments are necessary to elucidate its roles in immune cells.

Activation of NK and NKT Cells during Experimental Colitis in CD26 Deficient Mice

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The CD26 molecule (dipeptidyl peptidase IV, DPP IV, EC 3.4.14.5) is a broadly expressed multifunctional transmembrane glycoprotein involved in different biological processes through its proteolytic activity as well as its role in T-cell activation. Based on recent findings, immunological abnormalities including the overproduction of proinflammatory cytokines and natural killer T cells activation, suggest a potential role of CD26 in the pathogenesis of chronic inflammatory diseases. In order to investigate the role of CD26 in the etiopathogenesis of colitis, an experimental murine model was established.

Aim

The aim of this study was to investigate the potential role of CD26 in the development and activation of hepatic and splenic natural killer (NK) and NK T (NKT) cells in experimental murine model of colitis induced in C57BL/6 and CD26 deficient mice.

Material and methods

In this study, male homozygous CD26 deficient mice generated on a C57BL/6 genetic background and wild-type C57BL/6 mice were used. Colitis was induced by administration of 3% (w/v) dextran sulfate sodium dissolved in drinking water with free access during 7 days. Changes in percentages of liver and spleen NK and NKT cells were analyzed by flow-cytometry (FACSCalibur, Becton Dickinson). Data were evaluated using the Sigma Plot Scientific Graphing System, Version 6.10. The statistical significance was tested using Mann-Whitney U-test. Differences were considered statistically significant for $p < 0.05$.

Results

The results of our study show a decreased percentage of spleen NK and NKT cells in the acute phase of the disease in CD26 deficient and C57BL/6 mice in comparison with healthy mice. In CD26 deficient mice, in the acute phase of the disease, percentages of spleen NK and NKT cells were increased in comparison with C57BL/6 mice. Furthermore, in the acute phase of the disease, in CD26 deficient mice, percentages of NK and NKT cells in liver were statistically significantly increased in comparison to healthy mice.

Conclusion

Our results suggest a possible modulating role of the CD26 molecule in the regulation of local and systemic innate immune response in the acute phase of colitis, which makes it important in the pathogenesis of inflammatory processes.

Analysis of satellite DNAs in the sequenced genome of *Tribolium castaneum* (Coleoptera)

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The genome of the red flour beetle *Tribolium castaneum* was recently sequenced and draft assembly that represent about 70% of the genome was produced. It has been proposed that “unknown” parts of the genome represent regions of highly repetitive DNA that could not be assembled. In silico analysis of the abundance and distribution of repetitive DNA revealed that approximately 30% of the assembled genome is composed of repetitive DNA (Wang et al, *Genome Biology* 2008, 9:R61). In assembled genome satellite DNAs build only 2,5%. This result is in contrast with experimental data which revealed that highly abundant (peri)centromeric 360 bp satellite makes up to 17 % of the genome. We will present our in silico and experimental analysis of newly identified satellite DNAs from reptig files that could not be assembled into scaffolds, as well as a comprehensive analysis of 360 bp satellite DNA described previously.

Stability of N-glycan profiles in human plasma

Gornik O, Knežević A, Pučić Maja*, Redžić I, Wagner J, Lauc G

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Almost all plasma proteins are glycosylated and their glycan parts can exist in various structural forms, resulting in different glycoforms of the same molecule. Glycan heterogeneity was shown to be connected to many diseases and glycosylation holds an unbelievable diagnostic potential due to main biological features and structural characteristics of glycans.

Recently we observed high biological variability of human plasma N-glycome at population level. Thus, it was of great importance to standardize analytical methods and examine the temporal constancy of N-glycome in human individuals before routine implementation of techniques that determine glycosylation changes in diagnostic laboratories.

Plasma samples were taken from 12 healthy individuals. The blood was drawn on seven occasions during five days. N-linked glycans, released from plasma proteins, were separated using hydrophilic interaction high performance liquid chromatography into 16 groups (GP1-GP16) and quantified. The results showed very small variation in all glycan groups, indicating very good temporal stability of N-glycome in individual. Coefficients of variation from 1.6% for GP8 to 11.4% for GP1, with average of 5.6%, were obtained. These variations were comparable to those due to limitations in the experimental methods when analytical procedure was tested for its precision.

We conclude that plasma N-glycome in each individual has very good stability which implies that it is under genetic regulation. Changes occurring in glycan profiles are consequence of environmental influences and physiologic responses and therefore carry good potential diagnostic value.

Micronuclei detection in muscle tissue : Microscopy vs flow cytometry

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Exposure to a variety of toxic compounds present in aquatic environment is inevitable for marine organisms. In order to evaluate a degree of induced damage in exposed organisms, biomarkers of exposure have been applied in biomonitoring programmes. Due to the reflection of damage to genetic material of organisms, biomarkers of genotoxic effects are at high priority. Micronucleus assay, as widely used test for determination of environmental genotoxicity, has served as an index of cytogenetic damage for over 30 years. More recently it has been applied in biomonitoring of marine environment. The microscopic scoring of micronuclei is a tedious and time - consuming procedure. Attempts have been made to facilitate micronuclei scoring by means of image analysis or flow cytometry. Advantages of the flow cytometric techniques are that results can be obtained in short time intervals and that frequency of micronuclei as well as DNA content in micronuclei can be measured. In this study the microscopic and flow cytometric measurements of micronuclei distribution in the tissue of the common marine bioindicator, mussel *Mytilus galloprovincialis*, after treatment with model pollutants were compared and discussed.

Stability of N-glycan profiles in human plasma

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α v β 3 Integrin Mediated Drug Resistance in Human Tongue Squamous Cell Carcinoma

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Integrin-mediated drug resistance is based on the adherence of cells to extracellular matrix proteins through integrins. Integrins are cell surface heterodimeric receptors that mediate cell-extracellular matrix adhesion. They influence cell survival by modifying cellular response to chemotherapeutic drugs through mechanisms such as inhibition of apoptosis, decreased cellular proliferation and alteration in the expression of differentiation-regulated genes. We have recently described in human laryngeal carcinoma cells a novel mechanism of multidrug resistance mediated by α v β 3 integrin, involving glutathione dependent increased ability of α v β 3 expressing cells to eliminate drug induced reactive oxidative species (ROS). In the present study we investigated whether similar mechanism exists in tongue squamous carcinoma cells (Cal27) which express a small amount of α v β 3 integrins, and Cal27-derived stable transfectants with increased expression of α v β 3 integrin. The cell clones were obtained by stable transfection of Cal27 cells with a plasmid expressing the β 3 subunit. Using flow cytometry we measured the expression of α v β 3 and α v β 5 in these cell lines in order to verify whether the expression of α v β 3 decreased the expression of other α v integrins due to the competition of β 3 for available α v. In one stable transfectant the expression of α v β 3 was increased but the expression of α v β 5 remained the same in comparison to parental cell line. The other stably transfected cell line showed an increase in α v β 3 expression and a moderate decrease in α v β 5 expression, due to the competition of β 3 for available α v in the cell. The sensitivity of Cal27 and Cal27-derived α v β 3 integrin expressing clones to anti-cancer drugs was determined using MTT assay. Our results showed that both Cal27-derived α v β 3 integrin expressing cell lines were resistant to cisplatin, doxorubicin and mitomycin C.

Since the resistance mechanism involved in drug-induced ROS toxicity of human laryngeal carcinoma cells with increased α v β 3 integrin expression is mediated by glutathione, it is now our aim to show whether or not this is also the case with Cal27 cells. Increased α v β 3 integrin expression can protect cells from various cytostatics, therefore measuring its expression in head and neck cancers could be an important indicator of tumor resistance to selected anti-cancer drugs and possible target for therapeutic implications.

Antioxidant properties of *Teucrium arduini* L. flower and leaf infusions

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Phytochemicals are extensively found at different levels in many medicinal plants. Numerous plants used in traditional medicine are effective in treating various ailments caused by oxidative stress, bacterial and/or viral infections. Previous research has shown that Croatian medicinal plants exhibit antioxidant activity. Croatia has approximately 500 varieties of endemic herbs, which are commonly used in traditional medicine. One of them is *Teucrium arduini* L, prepared as an infusion, it is used in treatment of stomach ailments. Antioxidant activity as well as total phenol (TP, Folin-Ciocalteu method) content of leaf and flower infusions of *Teucrium arduini* L. from six different mountainous localities (Učka, Vošac, Sveti Jure, Snježnica, Vaganac, Sušanj) were analyzed in this study. Antioxidant capacity was evaluated using the ferric reducing/antioxidant power (FRAP) assay, as well as 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 2,2'-azinobis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) radical scavenging assays.

Randomly selected samples of wild growing plants *T. arduini* were collected during the blooming period in Croatia in the summer of 2008 and were air-dried and saved until analysis. Infusions were prepared on the day of measurement by adding 2 g of dried powdered plant material to 30 mL of deionised water heated to 95°C. The extraction proceeded for 8 minutes in a closed plastic vial at room temperature, with periodic stirring. Flower and leaf infusions from Učka showed the highest TP content and exhibited the highest reducing power. Our results point to significantly ($p < 0.05$) different TP content between leaf and flower infusions, as well as across localities. The DPPH• radical scavenging ability of leaf infusions can be ranked as follows: Vošac > Učka > Sveti Jure > Snježnica > Sušanj > Vaganac and that of flower infusions: Učka > Vošac > Sveti Jure > Sušanj > Vaganac > Snježnica. The flower infusions from Učka (8.47 ± 0.33 mmol/L TEAC) and Vošac (8.44 ± 0.43 mmol/L TEAC) showed the best ABTS•+ scavenging efficiency, while among leaf infusions the sample from Učka exhibited the highest antiradical activity (6.46 ± 0.67 mmol/L TEAC).

The antioxidant potency composite index (ACI), giving equal weight to all three methods used to quantify antioxidant capacity, was the highest for the infusion from Vošac (96.7) among flower infusions, while maximum ACI (100) was determined for the infusion from Učka among leaf infusions. Strong positive correlation was found between the total phenols and ACI for leaf ($r = 0.953$) and flower ($r = 0.977$) infusions indicating that polyphenolic content is highly correlated with the overall antioxidant capacity of *T. arduini* infusions.

Analysis of nucleosome arrangement on *Tenebrio molitor* satellite DNA

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The nucleosomal arrangement on satellite DNA of *Tenebrio molitor* beetle (Tenebrionidae, Coleoptera) has been studied. A single satellite DNA constitutes about half of the *T. molitor* genome. Very regular nucleosomal ladder consisting of about 170 bp long multimers was obtained after digestion of crude chromatin extract with micrococcal nuclease. All ladder rungs hybridized intensively with the satellite DNA probe, indicating uniform distribution of satellite-DNA bearing nucleosomes in the digested material. From the total population of fragments, satellite-containing fragments were selected after cloning and hybridization, and nucleotide sequences of these clones were determined. Sequence analysis indicates that micrococcal nuclease susceptible sites defined by nucleotide sequence were cleaved in preference, and assumably disregarding the nucleosomal positions. This effect might be a consequence of an extremely dense packing of nucleosomes in *T. molitor* heterochromatin because of putative tightly bound additional protein components, that remain under the isolation procedure. To circumvent this problem we are currently isolating core DNA fragments prepared from *T. molitor* nuclei using micrococcal nuclease, exonuclease III and nuclease S1.

SSB protein from *Streptomyces coelicolor* and its interactions with other proteins

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Single stranded DNA binding proteins (SSBs) are essential proteins in the cells of all living organisms. These proteins are involved in DNA recombination, replication and repair. Crystal structure of SSB from the multicellular spore producing bacterium *Streptomyces coelicolor* has recently been solved. This structure seems to be the most stable in comparison to other structurally characterized SSBs. SSB proteins from most prokaryotic species exist as tetramers which binds to ssDNA through structurally conserved folding motifs (OB folds). SSB proteins also possess conserved C-terminal domain which has role in interacting with other proteins. It has been shown that SSB protein from *Escherichia coli* interact with at least 14 different proteins.

There is no data on interactions of the *S. coelicolor* SSB with other cell proteins. Therefore, the aim of this study was to identify proteins that could interact with the SSB during the growth of the *S. coelicolor*. As a part of our deep interest to understand tyrosine phosphorylation of SSB protein and biological significance of SSB modification we have special interest in identifying tyrosine kinase involved in this process. TAP (tandem affinity purification) technology has been used to purify SSB and its interacting partners from *S. coelicolor*. This two step purification allows recovery of the protein complexes under native conditions. Sequences coding for two tags (protein A and Calmodulin binding peptide, CBP) separated with TEV protease cleavage site have been cloned on 5' terminus of *ssb* gene previously inserted into sequencing vector pGEM-T. Such construct was transformed into *S. coelicolor*, and protein complexes formed of SSB and unknown interacting partners were isolated from liquid culture in exponential phase of growth. The cell extract has been passed through IgG sepharose column which specifically binds to protein A. Bound recombinant protein was cleaved with TEV protease and applied to Calmodulin sepharose which binds CBP in presence of Ca²⁺ ions. SSB and its interacting proteins were eluted with the addition of chelating agent EGTA. Proteins were precipitated with TCA and separated with preparative SDS-PAGE. Nine visible bands were excised and extracted from the gel slices and mass spectrometry analyses has been performed.

Cultivation of human mesenchymal stem cells in animal serum free conditions

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Human mesenchymal stem cells (hMSCs) are nonhematopoietic multipotent stem cells. Although they are present in various organs and tissues they are mainly residing in the bone marrow (BM). MSCs have been shown to express immunomodulatory properties in vitro, like inhibiting T-cell proliferation after stimulation by alloantigens and mitogens and by preventing the activity of cytotoxic T cells. Since they are also immunoprivileged cells that could be transplanted across MHC barriers, their considerable therapeutic potential has generated increasing interest in the field of cellular therapy. So far, hMSCs have been clinically applied to facilitate engraftment in hematopoietic stem cell transplantation (HSCT) and to treat acute graft versus host disease (aGVHD). Since incidence of hMSCs in BM is only ~1 per 10⁴ nucleated cells, ex vivo expansion of these cells is necessary. Clinical application requires 2x10⁶ hMSCs/kg per adult patient. So far the most successful culture conditions for hMSCs expansion ex vivo included use of fetal bovine serum (FBS), which represents the potential risk of prion transmission and immunological reactions. For these reasons, there is a great interest in search for an FBS substitute and platelet lysate (PL) has been recently proposed.

The aim of this study was to evaluate the hMSCs cultured in FBS-free culture conditions.

Mononuclear cells (MNC) were isolated from BM sample of healthy donor with a Ficoll density gradient centrifugation and plated at 100 000/cm² in α MEM culture medium containing 10%FBS or 5%PL. PL was obtained from a single allogenic platelet unit prepared by the Hospital Transfusion Center. At subconfluency, cells were harvested after Trypsin-EDTA treatment and subsequently replated at 1000 hMSCs/cm² until reaching passage 5. To evaluate the clonogenic potential of cultured hMSCs colony-forming unit-fibroblast (CFU-F) assays were performed at each passage. The characteristic immunophenotype was assessed by flow cytometry. To assess their multipotential capacity, hMSCs were induced into adipogenesis at passage 3. hMSCs were also analyzed for chromosomal stability at passages 4 and 5 to validate their safety for potential use as a cell therapy product.

Our results showed that hMSCs were successfully cultured in media supplemented with PL. hMSCs cultured in that condition demonstrated lower increase in cell number and CFU-F colonies compared to cells cultured in the presence of FBS. Therefore the addition of bFGF (basic fibroblast growth factor) should be considered in the future expansion experiments. hMSCs expanded in PL condition expressed all known characteristics of cultured MSCs; adherence to plastic, expression of CD105 and

CD90 and in specific culture condition they were able to differentiate into adipocytes. Although most of analyzed metaphases in passage 4 had a normal karyotype, some of metaphases carried trisomy 8 which was not detected in following passage (p5). Karyotype anomalies are already described in the literature and since they are not stable and sustainable in later passages they appeared not to have selective advantage in the culture system and therefore are not of pathological nature. This study provides a basis for further efforts in evaluating PL as a valuable FBS substitute in clinical grade hMSCs expansion.

Mechanisms of Nuclear Envelope Breakdown In One Cell Stage *C. elegans* Embryos

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Nuclear envelope breakdown (NEBD) is a key event in the early stages of mitosis in all metazoans. In human cells, NEBD is thought to be facilitated by microtubules emanating from centrosomes, but it remains unclear whether centrosomes and motor protein dynein influence NEBD in a developing organism. Here, we used *C. elegans* one cell stage embryos to precisely analyze the mechanisms underlying the onset of NEBD.

Our results indicate that, although not essential, centrosomes influence the onset of nuclear envelope breakdown. Furthermore, we established that centrosome function in nuclear envelope breakdown is not mediated by dynein. Together, our findings suggest a model in which centrosomes, when present, influence NEBD in vivo by a microtubule-independent mechanism.

Molecular dynamics simulations of ligands binding in the active site of human dipeptidyl-peptidases (DPPIII)

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Dipeptidyl-peptidases III (peptidase family M49) are zinc-dependent enzymes that specifically cleave the first two amino acids from the N terminus of different length peptides. This zinc-dependent enzyme has been recently recognized among metallopeptidases, based on the unique structural motif, hexapeptide HELLGH, which harbours the predicted active site residues. Besides its contribution in normal protein catabolism, the regulatory and pathophysiological role for DPPIII was suggested, however molecular mechanism of its action is still unknown.

Recently determined crystal structure of human DPPIII (PDB code 3FVY) enabled detailed molecular modelling study. In order to understand mechanism of substrates (Arg-Arg-2naphthylamide and Ala-Ala-2naphthylamide) and inhibitor (Tyr-Phe-hydroxamate) binding into the active site of H-DPPIII we performed molecular modelling study using the Amber10 program suite. The initial structures were built in program Insight II (<http://accelrys.com/products/insight/>) and the steered molecular dynamics simulations were performed to determine possible orientations of the ligands in binding site.

We have determined similar binding of the ligands (inhibitor and substrates) into the DPPIII active site. Namely the hydrogen bonds formed during molecular dynamics simulations are mostly conserved in all three complexes. The zinc-binding site is built up by His-450, Glu-451 and His-455 belonging to the first conserved (450HELLGH455) signature motif, Glu-508 which is part of the second conserved motif (507EECRAE512), water molecule and carbonyl group belonging to the ligand second peptide bond from N terminus.

Longer simulations aimed to study stability of these complexes and to determine effects of amino acids mutations on ligands binding have been started.

Loss of heterozygosity of selected tumor suppressor genes in human testicular germ cell tumors

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Human testicular germ cell tumors (TGCT) are histologically heterogeneous group of neoplasms with variable malignant potential. Two main types of germ cell tumors occur in men: seminomas and nonseminomas, later generally having poorer prognosis. In the present study a set of tumor suppressor genes (CDH1, APC, p53 and nm23-H1) was investigated in testicular cancer for loss of heterozygosity (LOH). Both seminoma and nonseminoma tissue samples were analysed by PCR using either restriction fragment length polymorphism or dinucleotide or tetranucleotide repeat polymorphism method. The results of our analysis showed that the most frequent change was LOH of p53, although allelic losses of APC and CDH1 were also observed. No consistent LOH has been observed in all of the tumor types, and overall incidence of LOH of tumor suppressor genes in TGCT has shown to be about 15%.

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