Name:

Bambousková, Monika

Poster Title:

Electron Microscopy on Isolated Membrane Sheets as a Tool for Studies of Membrane-bound Activation Events

Authors:

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Abstract:

Ultrastructural analysis of isolated plasma membrane (PM) sheets using transmission electron microscopy (TEM) is a powerful technique for understanding the topography and organization of PM-bound signaling molecules. Specific probes are used to label receptors and signaling proteins on the extracellular and cytoplasmatic side of native membrane sheets prepared from live cells. Cells can be stimulated in various ways before isolation that gives an opportunity to follow up changes during cell activation. We use mast cells as a model for studying immunoreceptor signaling. These cells have long been recognized for their role in the genesis of allergic inflammation, and more recently for their participation in innate and acquired immune responses. Activation of mast cells is triggered by binding of a multivalent antigen (Ag) to specific IgE anchored to the high affinity receptors for IgE (FcεRI). This step initiates a signaling cascade that results in formation of multicomponent signaling complexes (signalosomes) on PM, further propagation of the signal and subsequent degranulation. The earliest known biochemical step that occurs after binding of multivalent ligand to the IgE-FcεRI complexes is tyrosine phosphorylation of the receptor subunits. However, the exact molecular mechanism of this phosphorylation is incompletely understood. We tested the hypothesis that changes in activity and/or topography of protein tyrosine phosphatases (PTPs) could play a major role. We found that exposure of rat basophilic leukemia cells or mouse bone marrow-derived mast cells to PTP inhibitors [H2O2 or pervanadate (Pv)], induced phosphorylation of the FcεRI subunits, similarly as FcεRI triggering. Interestingly, and in sharp contrast to FcεRI-induced activation, neither H2O2 nor Pv induced any changes in the association of FcεRI with detergent-resistant membranes (DRMs). For detail analysis of FcεRI distribution we focused on the topography of FcεRI on membrane sheets as detected by electron microscopy. Membrane sheets were isolated from nonactivated or activated mast cells using simple procedure (1-7). We have confirmed our previous data that exposure of the cells to multivalent antigen induced formation of FcεRI aggregates as detected by an antibody recognizing cytoplasmic chain. In contrast, we did not find any receptor clusters portion of the FcεRI after exposure of the cells to Pv. In cells stimulated with Pv, H2O2 or antigen, enhanced oxidation of active site cysteine was detected in several PTPs. Unexpectedly, most of oxidized phosphatases bound to the plasma membrane were associated with cytoskeleton-like structures. Experiments with biotin-labeled phalloidin, followed by gold-labeled streptavidin, and electron microscopy analysis showed that oxidized phosphatases colocalize with actin cytoskeleton. Based on these and other data we propose that down-regulation of enzymatic activity of PTPs and/or changes in their accessibility to the substrates play a key role in initial tyrosine phosphorylation of the FcεRI and other multichain immune receptors.

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Name:
Baresova, Petra

Poster Title:
Kaposi's sarcoma-associated herpesvirus-encoded nuclear antigen vIRF-3 targets tumor suppressor p53

Authors:
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Abstract:
Kaposi's sarcoma-associated herpesvirus (KSHV) is the causative agent of Kaposi's sarcoma, multicentric Castleman's disease and primary effusion lymphoma (PEL). The virus encodes a nuclear protein, viral interferon regulatory factor-3 (vIRF-3/LANA2), which is constitutively expressed in latently infected PEL cells. The main goal of our study is to understand the mechanism through which vIRF-3 inhibits the function of a key tumor suppressor gene, p53. Using co-immunoprecipitation and GST pull-down analyses we show that vIRF-3 associates with p53 both in vitro and in vivo. Furthermore, ectopic expression of vIRF-3 in HEK293 cells resulted in reduced p53 protein levels as well as down-regulation of p53 acetylation of lysine K320 after treatment with DNA damaging agent, etoposide. A detailed sequence analysis of vIRF-3 protein identified three potential binding sites with HAUSP/USP7 protein, which is a major p53-sepcific deubiquitinating enzyme. The consequence of direct binding between p53 and HAUSP is deubiquitination and subsequent stabilization of p53 protein (Li, et al., 2002). Our current research is focused on further functional characterization of vIRF-3-p53-HAUSP interaction. To further delineate the role of vIRF-3 in the inhibition of p53 function, we employed RNA interference to specifically knock-down vIRF-3 expression in PEL cells. PEL cell lines with specific down-modulation of the vIRF-3 gene expression would allow us to study the vIRF-3 function in the context of the entire KSHV genome in its natural cell host. Out of a pool of potential vIRF-3-specific siRNAs, we selected four with approximately 60 - 70% knock-down efficiency. The construction of PEL cell lines with specific knock-down of vIRF-3 is currently in progress. We believe that this comprehensive analysis will unambiguously establish the oncogenic potential of vIRF-3 and provide a new understanding of the mechanism by which virus-encoded vIRF-3 modulates the function of p53 tumor suppressor. This work was supported by the Academy of Sciences of the Czech Republic (Grant IAA501050701) and the Grant Agency of the Czech Republic (Grant 204/09/0773).

Name:
Bhattacharyya, Tanmoy

Poster Title:
Unsynapsed chromatin, meiotic transcriptional silencing and intrameiotic arrest of spermatogenesis in mouse interspecific hybrids

Authors:
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Abstract:
Hybrid sterility is a reproductive isolation mechanism limiting gene flow between related species. Two major genetic hybrid sterility loci/genes, Hst1/Prdm9 on Chr 17 and Hstx1 on Chr X, have been identified in sterile hybrid male mice from crosses between inbred strains PWD/Ph and C57BL/6J (B6) derived from Mus m. musculus and Mus m. domesticus subspecies, respectively (Mihola et al. Science 323:373,2009; Storchova et al. Mamm. Genome 15:515, 2004). Here we studied the molecular phenotype of intrameiotic arrest in FACS-sorted primary spermatocytes of (PWD X B6)F1 sterile males. The H1t-negative primary spermatocytes arrested at early-mid pachytene stage of meiosis formed 55% of testicular cells. Immunostaining of synaptonemal complex proteins SYCP1/SYCP3 disclosed partial autosomal asynapsis in up to 80% of pachytene cells. These cells displayed the yH2AX histones over the asynapsed autosomes and disrupted sex-body formation. Immunostaining of ATR and H3K9 indicated abnormal meiotic silencing of unsynapsed chromatin (MSUC). The arrested spermatocytes underwent apoptosis. The microarray (Affymetrix) profiling of gene expression in sterile and fertile hybrids revealed dysregulation of the X chromosome genes and significant downregulation of a fraction of autosomal genes in sterile prepubertal (16 dpp) males.

Name:
Bondar, Alexey

Poster Title:
Two-photon polarization microscopy: a novel tool for monitoring membrane protein function in live cells

Authors:
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Institute of Physical Biology

Abstract:
Membrane proteins are a large group of proteins with diverse cellular functions. Their inherent requirement for a lipid membrane makes membrane proteins difficult to study. We have recently developed a method of two-photon polarization microscopy (2PPM) that takes advantage of membrane localization of proteins, and allows observing protein-protein interactions, as well as conformational changes in protein molecules, in living cells, in real time. Our results show that 2PPM allows observing interactions among G protein subunits and G protein-coupled receptors, and monitoring G protein activation. Importantly, 2PPM requires only a single fluorescent tag, allows facile multiplexing, and yields insights about structural changes in protein molecules. A combination of these factors makes 2PPM a promising tool for studies of membrane protein function in live cells.

Name:
Černá, Hana

Poster Title:
NMR Study of Nrd1 CID in Complex with Ser5-phosphorylated CTD of RNA Polymerase II

Authors:
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Affiliations:
NCBR, Masaryk University

Abstract:
Many non-coding RNA transcripts of RNA polymerase II are processed by the poly(A) independent termination pathway that requires the Nrd1 complex. The Nrd1 complex consists of two RNA-binding subunits, the nuclear pre-mRNA down-regulation (Nrd)1 and the nuclear polyadenylated RNA-binding (Nab)3 proteins, and the RNA helicase Sen1. Transcription termination mediated by the Nrd1 complex involves a combination of its interactions with the C-terminal domain (CTD) of RNA Polymerase II phosphorylated at Serine 5 and with specific RNA sequences that occur in the nascent transcripts.
To reveal structural and mechanistic pictures of this concerted recognition mechanisms of Nrd1, we use nuclear magnetic resonance (NMR) spectroscopy and other biophysical tools. Based on our results, we will show how the structure of CTD is modulated by phosphorylation at serine 2, 5, and 7, respectively, and how the Nrd1 CTD-interaction domain (CID) recognizes Ser5-phoshorylated (Ser5P) CTD. Furthermore, we will show our NMR studies of the Ess1 prolyl isomerase acting on Ser5P-CTD and Ser5P-CTD bound to Nrd1 CID that provide an insight into the mechanism of the initial step in the Ser5P-CTD dephosphorylation.

Name:
Černý, Martin

Poster Title:
Novel links between temperature, calcium and cytokinin signaling identified by proteome and phosphoproteome-wide profiling in Arabidopsis

Authors:
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Abstract:
Cytokinins (CKs) regulate diverse developmental and physiological processes in plants whose molecular mechanisms of action are being intensely researched. Previously, we identified a number of early cytokinin response proteins and phosphoproteins of Arabidopsis thaliana. They indicated novel links between temperature and cytokinin signaling, and an involvement of calcium ions in cytokinin signaling. Here, we present results of further proteomic experiments aimed to bring a closer insight into these cross-talks. Using sub-proteome and phosphoproteome profiling, we identified early cytokinin response proteins and phosphoproteins whose regulation by cytokinins is abolished in the presence of calcium signaling inhibitors and/or altered under cold or heat shock.
Supported by grants IAA600040701, LC06034, 1M06030, GACR 206/09/2062, AV0Z50040507, AV0Z50040702 and AV0Z40310501.

Name:
Dobes, Pavel

Poster Title:

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Abstract:
ABSTRACT NOT AVAILABLE AT THE TIME OF PRINTING

Name:

Dolezalova, Dasa

Poster Title:

Changes to Cell Cycle Regulating Proteins and microRNAs in Differentiation of Human Embryonic Stem Cells into Neural Lineage

Authors:

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Abstract:

Human embryonic stem cells (hESCs) show specific cell cycle properties, such as short doubling time, abbreviated G1 phase, and stable expression of several cell cycle regulating molecules. Interestingly, all of these characteristics are rapidly changed with the onset of differentiation. Therefore it has been hypothesized, that cell cycle regulating molecules, which primarily function in regulation of cell cycle progression, can also participate in the process of differentiation as well as maintenance of particular differentiated phenotype.

We aimed to assess complex changes in configuration and activities of cell cycle regulators as well as cell cycle characteristics associated with molecular switch between self-renewing population of hESCs and proliferating neural progenitors/neural stem cells (NP/NSC) and neural crest precursor cells (NCPCs) derived from hESCs.

We show that particular cell cycle features are changed with the onset of differentiation while some of these changes might be specific only for the neural lineage commitment or even for particular NP/NSC or NCPC line. Interestingly, in addition to other changes, level of cyclin E was elevated prominently in differentiated NCPCs as well as NP/NSCs lines in comparison with hESCs. This effect might be specific for particular pathway of differentiation as such increase was, to our knowledge, observed in differentiation to neural lineage in Drosophila. We also show that stability of configuration of cell cycle regulating machinery in NP/NSCs line during longterm cultivation might be compromised as level of regulatory molecules (such as cyclins A and B) decreases during the prolonged cultivation of NP/NSCs. Moreover, using microarrays (LNA spotted oligos, EMBL, 470 miRNAs), we identified a set of microRNAs (n~30), whose expression is dramatically changed with differentiation (10-20 fold). Interestingly four miRNAs strongly over-expressed in hNPs – miR-21 (indirect regulator of CDK4/CDK1 via p21 and PDCD4), miR-221/miR-222 (direct regulators of p27) and miR-125b (regulates p53) have been previously annotated to cell cycle deregulation in cancer cells.

Our data provide an insight into configuration and changes in molecular mechanisms that accompany process of differentiation of hESCs into specific cell type and point out the crucial role of molecules regulating cell cycle progression in differentiation.

This study was supported by: MSM 0021 6224 30, AV0Z50390512, AV0Z50390703, 1M0538, LC06077, LSHG-CT-2006-018739, MUNI/G/0132/2009

Name:
Du, zhengyu

Poster Title:
Effect of gut bacteria from IBD patients on gnotobiotic mice

Authors:
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Institute of microbiology, Laboratory of Physiology, Immunity and Ontogenesis of Gnotobionts

Abstract:
Background: The pathogenesis of inflammatory bowel diseases is a combination of genetic, environmental and immunological factors. Experimental data support the important role of gut microbiota in the onset and persistence of inflammation.
Aims: (1) to investigate how microbiota taken from patients suffering from ulcerative colitis (UC) affect the development of dextran sulfate sodium (DSS)-induced intestinal inflammation in germ-free (GF) mice. (2) to compare bacterial composition from human biopsies to bacterial composition in cecum of colonized mice before and after DSS treatment. .
Methods: Biopsies were taken from descending colon of UC patients, homogenized and transferred into GF mice in anaerobic conditions. Mice were treated with 2.5% DSS for one week after three-month colonization. Conventional (CV) mice served as controls. Colitis was quantified by clinical scores, histological evaluation and myeloperoxidase activity. Cytokines were measured in supernatants of cultivated spleen cells by ELISA. Bacterial analysis was performed by denaturing gradient gel electrophoresis, DNA sequencing and aerobic and anaerobic cultivations.
Results and Conclusion: Colonization with microbiota of patients' biopsies did not induce colitis in ex-GF mice neither with nor without DSS treatment. DSS-induced colitis was severe in CV mice. High numbers of mucosal-associated bacteria were detected in cecum of colonized mice. For better understanding of the mechanism in host-microbiota interactions, further research needs to be performed.

Name:
Dvořáková, Eva

Poster Title:
Production of monoclonal antibodies against human prion protein

Authors:
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Institute of Immunology and Microbiology, First Faculty of Medicine, Charles University

Abstract:
Transmissive spongiform encephalophthies (TSEs) are neurodegenerative diseases characterized by deposition of abnormally folded prion protein (PrPres) in brain. PrPres is at present the only biochemical marker of human and animal TSEs. Diagnostic tests are based on detection of PrPres after proteinase K digestion of brain homogenate using Western blot or on immunohistochemistry of fixed brain tissue, which are both difficult and time consuming. In brains of patients with Creutzfeldt-Jakob disease (CJD) were shown increased levels of glycated prion aggregates. The role of glycation in PrPc-PrPres conversion is poorly understood. Advanced glycation end-products (AGEs) probably stabilize prion deposits. Glycated prion aggregates could represent another specific TSE marker and thus allow development of new and simpler diagnostic tests using monoclonal antibodies against PrP-AGE. The aim of this study was to prepare two types of monoclonal antibodies: first type directed against native human PrP and second type directed against modified human PrP. Recombinant human prion protein (rhPrP) was isolated from transformed E. coli BL21 (DE3) and purified on Cu resin (TALON) by affinity chromatography. rhPrP was modified by glyoxylic acid, that introduces carboxymethyl groups on lysines present within the molecule of prion protein. Modified rhPrP (rhPrP-AGE) was used for immunization of 9 mice. Splenic cells from mice producing highest titers of PrP antibodies were used for fusion with myeloma cell line resulting in production of 960 hybridoma cells. Clones were primary screened by ELISA method for reactivity to rhPrP and rhPrP-AGE. 53 selected clones were tested in secondary screening and 19 in tertiary screening by Western blot and dot blot on biological material (human and mouse brain and blood cells). Two clones with affinity to native hPrP were chosen for preparation of purified monoclonal antibodies and are commercially available by EXBIO as EM-20 and EM-21. They are being tested in National Reference Laboratory TSE-CJD in Thomayer University Hospital in order to replace confirmatory expensive antibody used in western blot and immunohistochemical preparations. The purified monoclonal antibody with affinity to hPrP-AGE (EM-31) displays no affinity to native non-modified PrP and has no cross-reactivity to other modified brain proteins. This antibody is now tested for reactivity with PrP in biological tissues of TSE infected and healthy subjects.

Name:
Flemr, Matyas

Poster Title:
Mouse oocyte growth is accompanied by reduction of microRNA activity and P-body disassembly

Authors:
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Abstract:
In mouse, a major portion of time during the oocyte-to-zygote transition occurs in the absence of transcription, and thus depends on post-transcriptional control of the maternal mRNA pool synthesized during oocyte growth. Many maternal mRNAs are stored in the cytoplasm and are translationally inactive. In somatic cells, the translationally repressed mRNAs are targeted to different RNA granules, such as stress granules or Processing bodies (P-bodies). P-bodies are cytoplasmic foci enriched in mRNA-destabilizing proteins, translational repressors and other RNA binding proteins, and microRNAs (miRNAs). To understand the regulation of maternal mRNA storage, recruitment, and degradation, we investigated the distribution of messenger-ribonucleoprotein (mRNP) complexes in mouse oocytes. We found that P-bodies disassemble early during oocyte growth and several P-body components, including RNA helicase DDX6 and polyadenylation regulator CPEB, form a novel type of mRNA storage granules in the cortex of fully-grown oocytes. Because P-bodies form as a consequence of miRNA pathway activity, we analyzed activity of maternal miRNA. We found that P-body disappearance correlates with reduced ability of let-7 and miR-30c miRNAs to repress translation although they are present and loaded on AGO2 in the oocyte. Furthermore, transcriptome analysis of oocytes lacking miRNA processing enzyme Dicer did not reveal any miRNA specific footprint in the set of differentially expressed transcripts. These data suggest that miRNA function is suppressed in mouse oocytes, perhaps in order to support mRNA-stabilizing environment of the oocyte cytoplasm and reprogramming of differentiated oocytes into pluripotent cells of the early embryo.

Name:
Ganji, Sri Ranjani

Poster Title:
Study of endogenous Dishevelled complex

Authors:

Affiliations:
Masaryk University

Abstract:
Wnt signalling pathways are evolutionarily conserved and regulates vital aspects of embryonic development in all animals. The interaction of Wnts with the seven pass transmembrane protein, Fzds and the coreceptors, LRP5/6 or Ror/RYK, activates the key component of Wnt pathway, Dishevelled (Dvl), that further triggers the down stream molecular components of the pathway. In the canonical wnt pathway, activated Dvl is recruited to Fzd which disintegrates the beta-catenin destruction complex, hence stabilizes cytosolic beta-catenin, which then translocates to nucleus. In case of non-canonical pathway Dvl interacts with RhoA via Daam1 hence activating planar cell polarity (PCP) signalling. Apart from this Dvl is also known to play crucial role in other non canonical pathways that include Wnt-calcium signalling, Wnt-RYK signalling and many more. To execute its versatile functions Dvl is known to interact with different set of proteins and enzymes. Composition of the complex varies depending on the functional state of Dvl. Main aim of this project is to understand endogenous Dvl function in Wnt induced pathways using proteomics approach. To contribute to the identification of Dvl regulators we performed pulldown of endogenous Dvl complex with and without Wnt stimulations combined with mass spectrometry. This led to detection of some of the known interactors of Dvl along with new Dvl interaction partners. Our goal is to study endogenous Dishevelledome in its basic functional state which provides key to the mechanism of how Dvl activates and directs individual branches of the Wnt signalling.

Name:

Glierova, Hana

Poster Title:

Comparison of binding of prion antibodies to lymphocytes and monocytes of cynomolgus monkey in peripheral whole blood and after their isolation using Ficoll

Authors:

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Abstract:

The essential event in pathology of prion diseases is conversion of cellular prion protein (PrPc) to its conformational isomer - infectious prion protein (PrPtse). PrPtse is the only component of the prion particle known so far and is necessary for the disease pathology. Four cases of transmission of prion disease by blood transfusion highlight the need for reliable screening tests for prions. Rather little is known about the nature and behavior of prions in blood, nevertheless, the infectivity in blood appears to concentrate in leukocytes. Nowadays, the only specific marker for prion diseases is PrPtse, but its detection is challenging as the PrPtse levels in peripheral tissues are quite low. The detection of PrPtse in blood is further complicated by the large amount of PrPc. Recently reported availability of several PrPtse specific antibodies brings to focus a possible flow cytometry detection of PrPtse on blood cells.

In this study we compared the expression of PrPc/PrPtse by peripheral blood leukocytes of healthy (n=6) and prion infected (n=5) cynomolgus monkeys and the influence of Ficoll separation of mononuclear blood cells on the detection of PrPc/PrPtse using quantitative flow cytometry. We employed three PE conjugated monoclonal antibodies (mAbs) against different epitopes of PrPc molecule: AG4 (31-51), 3F4 (109-114) and AH6 (159-175). In peripheral blood we detected moderate levels of prions on lymphocytes and monocytes while only negligible expression by granulocytes corresponding with our findings in human leukocytes. Two out of three mAbs used – 3F4 and AG4 – detected significantly less PrPc on Ficoll separated washed lymphocytes ($P<0.01$) suggesting the existence of a loosely-bound pool of PrPc which can be removed from the cell surface by washing. Analogical behavior of PrPtse would further complicate the detection of prions in blood.

Our findings reveal an interesting phenomenon which should be considered for the development of blood based assays for prions and for safety of blood derived products in transfusion medicine.

The study was supported by the Aliance BioSecure Research Foundation and by the grants NS10335-3 of Czech Ministry of Health and GAUK 86408 of Charles University in Prague.

Name:

Hašová, Martina

Poster Title:

Photoprotective effects of hyaluronan after acute and chronic UV induced damage of keratinocytes and fibroblasts

Authors:

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Affiliations:

1- CPN s r.o, Dolni Dobrouc, Czech Republic, 2-Institute of Biophysics, Academy of Sciences of the Czech Republic, Brno, Czech Republic

Abstract:

In this study we investigated whether hyaluronan (HA) influences keratinocytes and fibroblasts after UVB irradiation. Acute and chronic exposure to solar ultraviolet (UV) irradiations leads to immunosuppression, photoaging and carcinogenesis. Hyaluronan is a linear naturally occurring polysaccharide formed from repeating disaccharide units of N-acetyl-D-glucosamine and D-glucuronic acid. HA is involved in several key processes, including cell signaling, wound repair and regeneration, morphogenesis, matrix organization and pathobiology. HA and its effects was tested in in vitro acute model of UVB-irradiated keratinocytes and chronic model of UVB-irradiated fibroblasts. Cells were irradiated by single or repeated doses of UVB light (280-320 nm), treated with HA and the effects on cell proliferation, cell production of interleukin (IL) 1α, IL-6, IL-8, soluble extracellular fragment of CD44 (sCD44std) and transforming growth factor (TGF)-β1 were evaluated. We also tested HA effects on matrix metalloproteinase (MMP)-9 activity, which is involved in extracellular matrix (ECM) remodeling during UVB irradiation. HA suppressed the UVB-induced decreased of cell viability in acute and chronic models. HA suppressed the acute UVB-induced pro-inflammatory protein release including interleukin (IL) 1α and IL-8. HA reduced UVB-mediated TGF-β1 and sCD44std production in both models. HA inhibits MMP-9 activity 24 h after UVB irradiation. Data indicate that HA had significant protective effects against UVB irradiation. According to our results, HA increased cell viability, provides anti-inflammatory properties and decreased negative effects on the remodeling of ECM after UVB irradiation.

Name:
Hnilicova, Jarmila

Poster Title:
HDAC1 modulates alternative splicing

Authors:
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Abstract:
Increasing evidence suggests that pre-mRNA splicing occurs co-transcriptionally and splicing decisions are largely made when the nascent RNA is still attached to chromatin; yet little is know about coupling between chromatin structure, transcription, and splicing. Here we show that HDAC1 activity links splice site choice to histone acetylation. Using splicing-sensitive microarrays, we identified a number of genes whose splicing was altered after HDAC inhibition and further analyzed one of the main targets, the fibronectin gene (FN1). Treatment with three different HDAC inhibitors or depletion of HDAC1 in HeLa cells had similar effects on FN1 splicing and resulted in alternative exon skipping. Importantly, this effect was reversed by rescue with mouse HDAC1 but not by a catalytically inactive mutant. Furthermore, splicing changes correlated with the level of histone acetylation of FN1. HDAC inhibition promoted elongation rate, which dramatically altered RNA polymerase II occupancy of the alternative exon. In addition, HDAC inhibition reduced association of SR proteins, main regulators of the FN1 alternative exon with the transcription unit. Taken together, our results indicate that HDAC activity modulates alternative splicing through dynamic regulation of chromatin structure that affects elongating RNA polymerase II and co-transcriptional association of splicing regulators. These data also suggest the importance of splicing factor interaction with the nascent RNA for splicing outcome.

Name:
Hrdý, Jiří

Poster Title:
DIFFERENT IMMUNOLOGIC PROPERTIES OF CORD BLOOD CELLS OF CHILDREN OF HEALTHY AND ALLERGIC MOTHERS

Authors:
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Abstract:
In last three decades, there was a huge increase of allergic diseases. Strong genetic background of allergies led us to compare properties of cord blood mononuclear leukocytes (CBML) of children of healthy and allergic mothers in the effort to reveal the possible prognostic signs of future allergy development. Lymphocyte proliferation was tested by thymidine incorporation. As allergies belongs to diseases with predominant Th2 immune response, we ascertained gene expression and production of typical Th1 (IL-2, INF-gamma) and Th2 (IL-4, IL-5, IL-6, IL-13) cytokines by real-time PCR and ELISA, respectively. Flow cytometry was exploited for comparing expression of cell surface activation markers (CD80, CD83, CD86) on dendritic cells (DC) of children of healthy and allergic mothers. Gene expression of subunits of IL-12 family cytokines (IL-12, IL-23, IL-27, IL-35), IL-6 and TNF was followed in DC by real-time PCR. CBML of children of allergic mothers pose higher both spontaneous and polyclonally stimulated proliferation activity in comparison with children of healthy mothers. Gene expression and production of Th2 cytokines in CBML followed tendency to an increase and those of Th1 cytokines to a decrease in children of allergic mothers. Furthermore, gene expression of IL-12 family subunits after LPS stimulation of DC was higher in children of allergic mothers in comparison to children of healthy mothers. It is possible to conclude allergic phenotype is apparent already on the level of cord blood cells. Higher lymphocyte proliferation activity and higher stimulation readiness of DC in cord blood of children of allergic mothers imply easier allergy induction. Cytokine environment with increased bias to Th2 response in newborns of allergic mothers could contribute to future allergy onset.
This work was supported by grants of Ministry of Education, Youth and Sports of the Czech Republic MSM0021620806 and Grant Agency of the Czech Republic 310/08/H077.

Name:
Chlebova, Katarina

Poster Title:
NF449 is a novel inhibitor of fibroblast growth factor receptor 3 (FGFR3) signaling active in chondrocytes and multiple myeloma cells

Authors:
Katarina Chlebova, Pavel Krejci, Shunichi Murakami, Jirina Prochazkova, Jiri Smutny, Anie Aklian, Lukas Trantirek, Vitezslav Bryja, Alois Kozubik and William R. Wilcox

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Masaryk University

Abstract:
FGFR3 receptor tyrosine kinase represents an attractive target for therapy due to its candidate role in several human disorders including skeletal dysplasia, multiple myeloma, and cervical and bladder carcinomas. By using molecular library screening, we identified a compound named NF449 based on its inhibitory activity towards FGFR3 signaling in cells. In cultured chondrocytes or murine limb explants, NF449 rescued FGFR3-mediated extracellular matrix loss and/or growth inhibition, which represent the two major cellular phenotypes of aberrant FGFR3 signaling in cartilage. Similarly, NF449 antagonized FGFR3 action in the multiple myeloma cell lines OPM2 and KMS11, as evidenced by NF449-mediated reversal of ERK MAP kinase activation and transcript accumulation of CCL3 and CCL4 chemokines, both of which are induced by FGFR3 activation. In a cell-free kinase assays, NF449 inhibited kinase activity of both wild-type and disease-associated FGFR3 mutant (K650E), in a fashion that appeared non-competitive with ATP. Our data identify NF449 as a novel antagonist of FGFR3 signaling, applicable for FGFR3 inhibition alone or in combination with inhibitors that target the ATP binding site.

Name:
Kaucka, Marketa

Poster Title:
Wnt/PCP signaling pathway in B-cell chronic lymphocytic leukemia (B-CLL)

Authors:
Kaucka, Marketa; Krejci, Pavel; Kotasova, Jana; Pospisilova, Sarka; Malinova, Karla; Prochazkova, Jirina; Mayer, Jiri; Kozubik, Alois; Bryja, Vitezslav

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Abstract:
Recently, we have found upregulation of the Wnt/PCP pathway components in the CD19+ B lymphocytes from patients suffering from chronic B-lymphocytic leukemia (CLL). Core components of the Wnt/PCP pathway have been identified in Drosophila wing and eye as regulators of cell polarity. Homologues of Drosophila PCP proteins are also known in vertebrates, where they were shown to regulate convergent extension movements during gastrulation, neurulation and to determine the polarity of hairy cells in the inner ear.
We show that expression level of the Wnt/PCP pathway components in human B cells from CLL patients correlates with disease stage, which suggests that PCP genes and proteins can serve as possible prognostic markers in CLL. Using human cell line Mec1 and human endothelial cell line HUVEC in migration assays, we suggest that PCP proteins regulate migration of CLL cells in the chemokine (CXCL12, CCL19 and CCL21) gradient. Such migration is promoted by Wnt5a, which is a soluble ligand of the noncanonical Wnt pathway, and is modulated by knockdown or overexpression of Wnt/PCP proteins both in primary human B lymphocytes and human cell line Mec1. Our data suggest that Wnt/PCP pathway can act as critical regulator of B-CLL cell migration and CLL pathogenesis.

Name:
Khabiri, Morteza

Poster Title:
influence of organic solvents on haloalkane dehalogenase DbjA:computational study

Authors:
Morteza Khabiri, Babak Minofar, Veronika Štěpánková, Radka Chaloupková, Jiří Damborský, and Rüdiger Ettrich

Affiliations:
Institute of Systems Biology and Ecology, Academy of Sciences of the Czech Republic, Zámek 136, 373 33 Nové Hrady 2Loschmidt Laboratories, Institute of Experimental Biology, Masaryk University, Kamenice 5A/4, 625 00 Brno

Abstract:
Enzymes never exist in vacuum, but either in crystalline, glass-transition, or solvated state, with the solvated state being the “functional” one and the primary solvent being a water/salt mixture. However, already in the 1970s the stabilizing effect of various organic solvents on a protein was described experimentally (1). Nevertheless, to date computational simulations focused mainly on modeling enzyme reactivity in water. Little computational work has been done with the aim to study the molecular reasons for the stabilizing or destabilizing effect of organic solvents and gain a fundamental understanding of its influence on protein structure-function relationships. In this study we have chosen DbjA from Bradyrhizobium japonicum USDA110, belonging to the haloalkane dehalogenase family that are able to cleave carbon-halogen bonds. As the enzymes come from bacteria that use halogenated organic compounds as their growth substrate, their interaction with organic solvents is not an artificial in vitro situation, but physiologically relevant. This enzyme exhibit a very different behavior in various organic solvents. For example isopropanol/water mixture dampens vibrational and rotational modes in the DbjA structure while formamide and acetone intense this behavior.Therefore a detailed analysis of the molecular interactions on the protein surface and in the solvent shell around the protein leads to a fundamental understanding and a generalization of the organic solvent effects on protein structure-dynamics-function.

Name:
Knitlova, Jarmila

Poster Title:
Histological Evaluation of Immune Response to Vaccinia Virus in Nc/Nga Mice

Authors:
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Affiliations:
Institute of Immunology and Microbiology, 1st Medical Faculty, Charles University

Abstract:
Vaccinia virus-based vaccine against smallpox is associated with several adverse side-effects. One of the complications is eczema vaccinatum, a disseminated vaccinia virus infection. This condition affects patients with atopic dermatitis (AD). Since the worldwide vaccination against smallpox was stopped in 1979, the understanding to the pathogenesis of eczema vaccinatum from the perspective of today's immunology is very limited.
We have decided to develop a mouse model of eczema vaccinatum, using spontaneously mutated Nc/Nga mice strain, which is prone to develop atopic dermatitis.
Nc/Nga mice together with control mouse strains Balb/6 and C57Bl/6J were epicutaneously sensitized with ovalbumin to induce allergic skin lesions. Nc/Nga mice show thickening of the epidermis and dermis, as well as parakeratosis, the findings similar to those observed in human AD patients. Mice were inoculated with vaccinia virus and the histological analysis of the skin was performed. Namely, inflammatory cells infiltration and development of satellite skin lesions was followed. The results of this analysis will be presented

Name:
Kobets, Tetyana

Poster Title:
A novel research and diagnostic tool for Leishmania parasite detection and quantification.

Authors:
Tetyana Kobets1, Jana Badalová1, Igor Grekov1, Irina Kurey1, Helena Havelková1, Milena Svobodová2, Marie Lipoldová1

Affiliations:
1. 1. Institute of Molecular Genetics AS CR, v.v.i., Czech Republic, 2. Faculty of Science; 2. Charles University, Prague, Czech Republic

Abstract:
Leishmaniasis in humans is a great health problem in a number of countries. Leishmania is a protozoan parasite that infects mononuclear phagocytes of a vertebrate host. Leismania infections cause a wide spectrum of clinical manifestations, with the outcome of cutaneous or a systemic visceral disease. The expansion of the parasites is determined by multiple factors, including both parasite characteristics and host genetics.
Spread and load of parasites is an important disease parameter. Detection and quantification of parasites is one of the central problems of parasitology and tropical medicine. To solve this problem, a lot of different methods were developed. Methods of parasite detection include microscopy, cell cultures, immunodetection and DNA-based techniques. At the present moment, PCR-based approaches are the most promising, but none of them is completely satisfactory: some steps still remain rather complicated than simple.
We developed a PCR-ELISA-based method for quantification of parasite DNA in total DNA isolated from organs and tissues. The biotin- and digoxigenin-labeled primers complementary to the 120 bp conservative region of kinetoplast minicircle DNA were used. The PCR product was quantified by sandwich ELISA using antibodies against digoxigenin. Our method is simple, highly sensitive, and allows analyzing a large number of samples in a short time. Present method can be widely used for research purposes as well as improve diagnostics in medicine. We applied this technique to study the effects of host genotype on the parasite spread in L. major infected mice and detected organ specific control of parasite load. Two loci were mapped in a cross between mice of BALB/cHeA and recombinant congenic strain CcS-11: Lmr20 on chromosome 1 controls parasite numbers in inguinal lymph nodes (as well as serum levels of IgE and IFNγ) and Lmr5 on chromosome 10 influences parasite load in spleen (as well as skin lesions, splenomegaly, and serum IgE and IFNγ level). Three additional loci also strongly influence the disease phenotype: Lmr21 (chr. 7) controls skin lesions and IFNγ level, Lmr22 (chr. 16) controls IFNγ level, and Lmr23 (chr. 19) controls skin lesion. These results show that parasite elimination and other immunological and pathological processes are under control of different sets of genes that overlap only partly.

Name:
Kucera, Jan

Poster Title:
Regulation of stem cell fate by redox modifying agents

Authors:
Jan Kucera1, Lukas Kubala2, Hana Kotasova1, Katerina Stefkova1, Jirina Prochazkova1, Jiri Pachernik1

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Abstract:
Embryonic stem (ES) cells provide a powerful tool for studying embryogenesis as well as potent source of pluripotent cells and differentiated progeny for use in regenerative medicine. Besides the well-documented LIF induced STAT3 activation, self-renewal and maintenanace of mouse ES undifferentiated phenotype appears to be under control of PI3K/Akt signalling pathway. Differentiation seems to be promoted by Ras/Erk cascade. A number of intrinsic transcription factors have been identified so far, among them Nanog and Oct3/4 play pivotal role in pluripotency regulation. There is a growing body of evidence that unique characteristcs of stem cells are also closely conncted to redox regulation. Under physiological conditions in their niche, stem cells are kept in hypoxic microenvironment. Leaving their niche they are exposed to normal levels of oxygen. Thus reactive oxygen species (ROS) formation in stem cells due to hypoxic conditions could be considered as important regulator of stem cell fate. Here we analyzed effects of inhibitors of NADPH oxidases apocynin or diphenyleneiodonium (DPI) and a glutathione precursor N-acetyl-cysteine (NAC) on maintenance of mouse ES cells. Inhibitors of NADPH oxidases and NAC decreased proliferation of ES cells without significant effects on cell cycle distribution. Apocynin strongly inhibited Akt that was accompanied with dowregulation of Nanog and Oct3/4 expression. Apocynin also promoted changes in morphology of cells towards more differentiated phenotype. By contrast DPI enhanced Akt activation. NAC did not modified STAT3, Akt or Erk. Obtained results are compared with effects on mouse ES cells cultured in hypoxic condition. Hypoxic cultivation markedly reduced Erk phosphorylation, with no effect on Akt and STAT3. It could be concluded that apocynin significantly modulate intracellular signaling pathways involved in maintenance of undifferentiated phenotype of mouse ES cells. However, the mechanism of these effects through the apocynin induced inhibition of ROS formation is questioned and remains to be clarified, as well as different response to apocynin and DPI treatment.
This work was supported by grant from Czech Science Foundation 301/08/0717
and 204/09/H058.

Name:

Kurey, Iryna

Poster Title:

Mouse genetic model for clinical and immunological heterogeneity of leishmaniasis

Authors:

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Affiliations:

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Abstract:

The outcome of leishmaniasis depends on biological characteristics of both the host and the infecting parasite strain. In order to study the response to Leishmania major infection we analysed mice derived from two inbred strains: BALB/cHeA (highly susceptible strain), and STS/A (strongly resistant strain). We genetically dissected clinico-pathological and immunological characteristics of L. major-induced disease using 20 recombinant congenic BALB/c-c-STS/Dem (CcS/Dem) strains that carry different random subsets of 12.5% genes of the donor strain STS on the BALB/c background, converting multigenetic differences between strains into oligogenic or single gene differences. Linkage analysis was integrated with the study of multiple manifestations of infection – including skin lesions, visceral pathology, parasite load and several immunological parameters – leading to the mapping of 21 new loci, Lmr3-23 (Leishmania major response), that are associated with different combinations of pathological symptoms and immunological reactions. Eight loci control both pathological and immunological parameters, twelve influence immunological parameters only. Moreover, the genetics of clinical symptoms is also very heterogeneous: loci Lmr4, Lmr13 and Lmr21 determine skin lesions only, Lmr5 and Lmr10 skin lesions and splenomegaly, Lmr14 and Lmr3 splenomegaly and hepatomegaly (Lmr3 also skin lesions), and Lmr15 hepatomegaly only. We found that parasite elimination, clinico-pathological and immunological characteristics are regulated by different subsets of genes that only partly overlap. In conclusion, these studies revealed a network-like complexity of the combine effects of the multiple functionally diverse QTLs and their individual specificity.

Name:
Kvíčalová, Markéta

Poster Title:
Magnetoreception of American cockroach is sensitive to intensity of UV light.

Authors:
Kvíčalová, Válková, Vácha

Affiliations:
Masaryk University

Abstract:
The question whether animals' ability to perceive geomagnetic field is light-dependent or not has been addressed since the beginning of the research of magnetoreception. The role of light is essential in the problem of still enigmatic mechanism of reception. In this study, we tested the magnetoreception of American cockroach (Periplaneta americana) under two UV light intensities and under the darkness. The reaction to magnetic pulses was significant when light of high intensity was applied, it neared the limit of significance in the low light and it vanished in darkness. This study, as the first on insects, specifies the level of light intensity where the magnetoreception is functional. Unlike previous conditioning approaches sensitive to unspecific motivation impacts of changed light regime, our assay makes use of spontaneous behavioral reaction thus pointing more directly to the light-dependent reception mechanism.

Name:
Laláková, Jana

Poster Title:
Dissection of functional domains within the TRAMP4 complex

Authors:
Jana Laláková, Marie Sárazová, Peter Holub, and Štěpánka Vaňáčová

Affiliations:
National Centre for Biomedical Research

Abstract:
TRAMP4 and TRAMP5 are heteromeric complexes that promote the degradation of aberrant and short-lived RNA substrates through interaction with the nuclear exosome. TRAMP4 consists of the poly(A) polymerase Trf4, the RNA-binding protein Air2 and the RNA helicase Mtr4. The common model predicts that TRAMP recognizes aberrant RNAs, adds short poly(A) tails that serve as a platform for the exosome-mediated RNA degradation. However, we, and others have shown that catalytically inactive TRAMP4 is sufficient to mediate degradation of most of its substrates in vivo. Our aim is to understand how TRAMP activates exosome in a poly(A) independent manner.
To investigate in detail the protein-protein and protein-RNA interactions within the complex and to assess the level of functional importance of distinct protein domains, we prepared a series of deletion and point mutants in Trf4 and Air2 proteins and expressed them in different genetic backgrounds. We identified mutants with severely impaired growth phenotype. Currently, we are analyzing selected mutants for RNA processing defects as well as for the integrity of the TRAMP complexes.

Name:
Legartová, Soňa

Poster Title:
Genetics and epigenetics of multiple myeloma

Authors:
Soňa Legartová, Eva Bártová, Gabriela Galiová and Stanislav Kozubek

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Abstract:
Multiple myeloma represents example of tumour cell transformation characterized by multiple genetic abnormalities and epigenetic changes. Karyotypic abnormalities of MM correlated with the stage of this disease and drug response. Here, we focused on genetics and epigenetics in MM cell lines. Using ChIP-PCR (chromatin immunoprecipitation) and MeDIP (methylated DNA immunoprecipitation) we have analyzed epigenetic profiles such as H3K9 acetylation and H3K9 di-methylation at promoters and coding regions of the c-myc and CCND1 genes. Finally, we optimized MeDIP for promoters of candidate genes. Our analyses should provide information how histone signature and DNA methylation can be affected by cytostatic treatment used by clinics. As example, analyzing H3K9 acetylation at selected promoter regions, we observed interesting differences between the control and melphalan treated myeloma cells. Our results showed that cytostatic drug, melphalan, has ability to increase H3K9 acetylation at specific genomic regions (Krejci J et al., 2009)

Name:
Liskova, Jana

Poster Title:
Cell death in vaccinia virus-infected cells

Authors:
Jana Liskova, Zora Melkova

Affiliations:
Institute of Immunology and Microbiology, First Faculty of Medicine, Charles University in Prague

Abstract:
In most cell types, including epithelial cell lines HeLa G and BSC-40, vaccinia virus infection is considered to cause a lytic cell death, an equivalent of necrosis,. However upon infection of HeLa G and BSC-40 cell lines with vaccinia virus strain Western Reserve, we have previously observed increased activation and activity of caspases, a typical sign of apoptosis (Kalbacova et al., 2008). Consequently, we decided to explore this unexpected finding in further detail. First, we characterized the activation of caspases by flow cytometry using fluorescent pan-caspase inhibitor FITC-VAD-FMK (CaspACE) which binds to the active centre of caspases, but does not bind to pro-caspases. Using non-fluorescent inhibitors specific for individual caspases (1, 2, 3, 4, 6, 8, 9, 10, 13) to compete out binding of the fluorescent FITC-VAD-FMK, we identified caspase-2, -4 and slightly -3 as the activated species. Further, we determined the activity of the three caspases in lysates of vaccinia virus-infected cells using specific fluorogenic substrates. In lysates of infected HeLa G cells, we observed the activity of all the three caspases, but in lysates of BSC-40 cells, there was no detectable caspase activity found. We then examined the changes of the cells in situ in a time course experiment focusing on late intervals after infection (24, 48, 72, 96 hours after infection, h.p.i.). We characterized in vivo the morphology and fluorescence of cell nuclei using Hoechst 33342 and the permeability of the plasma membrane using propidium iodide. In late times after infection (48 h.p.i. and later), the nuclei of infected HeLa G cells displayed mostly apoptotic morphology (condensed and fragmented nuclei), while morphology of the nuclei of BSC-40 cells remained unchanged. The permeability of the plasma membrane increased at late h.p.i. in infected BSC-40 cells, but not in HeLa G cells. We also determined another hallmark of apoptosis - activity of the executioner caspase-3 in vivo using a fluorogenic cell-permeable substrate. Infected HeLa G cells revealed a high fluorescence, whereas infected BSC-40 cells were only slightly positive. Additionally, we proved the activity of executioner caspases in HeLa G cells by detecting the cleavage of the death substrate PARP using western blot analysis.
Based on these results, we conclude that HeLa G cell line infected with vaccinia virus strain Western Reserve displays morphological and biochemical hallmarks of apoptosis, which is contradictory to the generally accepted concept. On the other hand, infection of BSC-40 cells appears as lytic, confirming previous findings. The type of cell death does not seem to affect vaccinia virus growth cycle or yields, but it could be an important factor in modulating immune responses against vaccinia virus infection in the host organism.

Name:

Martinková, Jiřina

Poster Title:

Proteomic profiling of cancer cells after anti-cancer treatment

Authors:

Jirina Martinkova1, Rita Hrabakova1, Petr Halada2, Marian Hajduch3, Hana Kovarova1

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Institute of Animal Physiology and Genetics AS CR

Abstract:

Monitoring of therapeutic response to anti-cancer drugs is of growing importance and a wide range of proteomic techniques is being utilised in searching for new and clinically relevant biomarkers. Cisplatin, anti-cancer drug used in a combination therapy of a number of solid tumors, was approved by the US Food and Drug Administration for the treatment in 1978. To overcome numerous side effects and drug resistance development after cisplatin treatment, other platinum-based compounds were studied. Carboplatin has similar activity as cisplatin, but is more stable and less toxic. Oxaliplatin has a slightly different activity and serves as a standard therapy for colorectal cancer.

Our study is aimed at studying molecular mechanisms in response to anti-cancer therapy. We are using separation proteomic techniques to detect proteins that may be responsible for early changes in the signalling networks and may be involved in irreversible anti-cancer processes. Human CEM T-lymphoblastic leukaemia cell line was used as a model of haematological malignancy and cells were treated with above-mentioned platinum-based compounds. We hope that this in depth investigation will generate comprehensive overview of ongoing processes in cells treated with different platinum compounds and that these findings will be quickly implemented into clinics to help to personalize patients therapy.

We acknowledge support from the Czech Ministry of School and Education (MSM6198959216 and LC07017) and Institutional Research Concepts AV0Z50450515 (IAPG) and AV0Z50200510 (IMIC)

Name:
Nejepinska, Jana

Poster Title:
Effects of long double-stranded RNA i mammals

Authors:
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Institute of Molecular Genetics, AS CR

Abstract:
Double-stranded RNA (dsRNA) can induce different effects in mammals. These may be either sequence-specific, such as post-transcriptional gene silencing mediated by RNA interference mechanism, or sequence-independent. The latter are mimicking the antiviral response, including translational inhibition and non-specific RNA degradation, and thus are known as 'interferon response'. In order to study diverse effects of long dsRNA, we expressed a long RNA hairpin with dsRNA stem of ~500 bp. Using this system, we have created transgenic and cell culture models, allowing the studies of aforementioned processes in context of a whole reproducing organism or in a system easily accessible for experimental interventions, respectively. We have shown that transcribed RNA hairpin enters RNAi pathway exclusively in mouse oocytes while it has no detectable effects in other tissues, where the transgene is active. In transiently transfected cells, RNA hairpin induces sequence-independent reporter silencing but has a minimal effect on the transcripts and reporters integrated in the genome. Our data indicate that somatic cells possess mechanisms preventing detrimental effects of dsRNA expressed in the nucleus and that such dsRNA is directed into RNAi pathway exclusively in oocytes, the cell type where endogenous siRNAs were reported. Furthermore, cell culture experiments show that mammalian cells might dispose of a novel pathway responding to dsRNA by silencing the expression from extrachromosomal DNA.

Name:
Panigaj, Martin

Poster Title:
Silencing of PRNP gene by RNA interference suggests that cellular prion protein is dispensable for erythroid differentiation in vitro

Authors:
Martin Panigaj, Hana Glierova and Karel Holada

Affiliations:
Institute of Immunology and Microbiology, 1st Faculty of Medicine, Charles University

Abstract:
Cellular prion protein (PrPc) is expressed in various murine tissues, however only neurons in brain seem to be affected upon infection with prions. Therefore most of the attention is focused on the role of PrPc in neurons, but despite the longtime effort the role of PrPc is still not known in any type of cell. There is an evidence that expression of PrPc may affect self-renewal and cell survival during haemathopoiesis or erythropoiesis, respectively. Moreover, disregulation of transcription of several erythroid genes during prion infection was already described. We and others found out that in a model of in vitro erythroid differentiation represented by murine erythroleukemia cells (MEL), PrPc is transcriptionaly upregulated. Interestingly, PrPc production is the highest on the beging of differentiation (24 hours post induction) and then decreases. The upregulation is specific for late commitment phase and is not suppressed by blocker of differentiation - dexamethasone. In search for the role of PrPc in the cell differentiation we created MEL lines with stable silenced expression of PrPc (LP1 and LP2). The model is based on the integration of retroviral vector expressing shRNA in context of miRNA. Inhibition of PrPc expression (~ 80-90 % in comparison with control LN line) is stable during 6 day course of differentiation. During the differentiation we observed similar level of total hemoglobine content and similar pattern in the expression of selected erythroid genes (α- hemoglobine stabilizing protein, hemoglobine α). Similarly cell kinetics and viability was comparable in all lines. Upon infection of all MEL lines with brain homogenate harbouring prion strain RML5 we observed by cell blot assay equal presence of PrPtse irrespective to PrPc silencing. It suggests that inoculum of RML5 is unspecifically attached to LP1 and LP2 lines and moreover is not propagated by control lines. Taken together our data suggest that PrPc may be dispensable for erythroid differentiation in vitro or its remaining expression (~10- 20%) sustain its role. The possible role of PrPc in MEL cells after exposure to exogenous stress conditions is subject of undergoing research. This work was supported by GAUK 203429, Grants of Czech Science Fundation 310/08/0878.

Name:

Petr, Tomáš

Poster Title:

Crucial factors for gangliosides detection

Authors:

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Abstract:

A comparison of histochemical detection of GM1 ganglioside in cryostat sections using cholera toxin B-subunit after fixation with 4% formaldehyde and dry acetone gave inconsistent and tissue-dependant results. In the liver neither pretreatment showed detectable differences relative to GM1 reaction products, while studies in the brain showed the superiority of acetone preextraction (followed by formaldehyde), which yielded sharper images compared to the diffuse, blurred staining pattern associated with formaldehyde. Therefore, the aim of our study was to define the optimal conditions for the detection of GM1 using cholera toxin B-subunit.

Ganglioside extractability with acetone was tested comparing anhydrous acetone with acetone containing admixture of water. TLC analysis of acetone extractable GM1 ganglioside from liver sections did not exceed 2% of the total GM1 ganglioside content using anhydrous acetone at –20 °C, and 4% at room temperature. The loss increased to 30.5% using 9:1 acetone/water. Similarly, photometric analysis of lipid sialic acid, extracted from dried liver homogenates with anhydrous acetone, showed the loss of gangliosides into acetone was only 3.0 ± 0.3%. The loss from dried brain homogenate was 9.5 ±1.1%.

Thus, anhydrous conditions (dry tissue samples and anhydrous acetone) are crucial factors for optimal in situ ganglioside detection using acetone pretreatment. This insures effective physical fixation, especially in tissues rich in polar lipids (precipitation, prevention of in situ diffusion), and removal of cholesterol, which can act as a hydrophobic blocking barrier.

Name:
Podolska, Katerina

Poster Title:
Development and validation of microRNA pathway inhibitors

Authors:
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Abstract:
MicroRNAs are noncoding RNAs inducing sequence-specific posttranscriptional inhibition of gene expression and are the major class of small endogenous RNAs in mammalian cells. Hundreds of microRNAs potentially regulating more than 60 % of mammalian genes have been identified. MicroRNAs participate in the majority of cellular processes and their expression changes in various diseases, including cancer. Currently, there is no efficient small chemical compound available for modulation of microRNA pathway activity. The main goal of this project is to find and functionally characterize a compound inhibiting the microRNA pathway. After establishing of high-throughput cell-based luciferase assay for screening of potential microRNA pathway inhibitors, we screened ~ 15,000 small compounds from different compound libraries. Based on the results of this primary screening, candidate inhibitors are currently under characterization by other in vivo and in vitro assays, including in vitro fluorescent Dicer assay. The most suitable inhibitors will be used to study the role of microRNAs in dynamically changing systems, such as early events in embryonic stem cells differentiation. An effective microRNA inhibitor will substantially change options for studying RNA silencing pathways and might have a therapeutic potential.

Name:
Polakovičová, Iva

Poster Title:
NTAL: a positive or negative regulator of mast cell signaling?

Authors:
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Abstract:
Non-T Cell Activation Linker (NTAL) is a transmembrane RI signaling in mast cells is not fully adaptor which function in the Fc understood. Previous studies done in bone marrow-derived mast cells (BMMC) from NTAL-/- mice showed NTAL as a negative regulator while diminution of NTAL expression by silencing RNA oligonucleotides in human mast cells showed NTAL as a positive regulator. We compared mouse NTAL+/+, NTAL-/- BMMC, and cells with reduced NTAL levels prepared by using lentiviral system to transfer shRNA into cells. Comparing these three kinds of cells we observed that NTAL-/- cells and cells with reduced NTAL expression behaved very similar. Both cells showed significantly enhaced secretory and calcium responses after antigen activation compared to WT cells. Activation-induced tyrosine phophorylations of LAT (linker for activation of T cells) and ERK were also enhanced in cells with reduced NTAL levels. These cells also exhibited reduced spreading after activation on fibronectin and rapid depolymerization of filamentous actin. Supported in part by KAN200520701 and 301/09/1826

Name:
Potměšil, Roman

Poster Title:

Authors:

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Abstract:
ABSTRACT NOT AVAILABLE AT THE TIME OF PRINTING

Name:
Rusnakova, Vendula

Poster Title:
HER-2 gene expression in primary tumor tissue and circulating tumor cells

Authors:
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Affiliations:
Institute of Biotechnology

Abstract:
Breast cancer is the most common cancer in women. In 20 % - 30 % of the patients the HER2 gene is amplified, what is correlated with the higher HER2 expression and finaly with the disease prognosis too. Determination of HER 2 status enables to start biological treatment with HER2-blocking antibody. We have compared HER2- gene mRNA expression in primary cancer tissue and circulating tumor cells in breast cancer patients.
In primary BC five ml blood was collected before and after chemotherapy (n=87 patients). In metastatic BC (MBC) 5 ml blood (n=72) was studied either at the time of relapse of BC or at a documented progressive BC before receiving new therapy CTCs were immunomagnetically enriched using the AdnaTest BreastCancerSelect (AdnaGen AG, Langenhagen, Germany) followed by RNA isolation, subsequent gene expression analysis by reverse transcription and multiplex-PCR for the detection of EpCAM, MUC-1 and HER-2 transcripts. RNA from formalin fixed paraffin embedded (FFPE)-tumor tissue (n=46) was isolated with RecoverAll® (Ambion). After reverse transcription the cDNA from CTC and FFPE was gene-specifically pre-amplified for multimarker qPCR analysis on the Biomark® (Fludigm, USA) microfluidic chip for 48 genes in each of 48 samples (2034 rxn in total). qPCR data were analyzed with GenEx ver. 5.0 (MultiD, SE) and correlated to available clinical data.
The analysis has shown that the gene expression profiles of CTCs in primary breast cancer patients relate to the gene expression profiles of primary tumor. Opposites the gene expression profiles of CTC in MBC differ from the primary tumor significantly.

Name:

Seifertová, Eva

Poster Title:

A Genetic Map of Xenopus tropicalis

Authors:

Dan E. Wells1, Laura Gutierrez1, Zhenkang Xu1, Vladimir Krylov2, Jaroslav Macha2, Kerstin P. Blankenburg3, Matthew Hitchens3, Lawrence Bellot1, Mary Spivey1, Andria Kowis3, Yuan Ye3, Shiran Pasternak3, Jeanetta Owen1, Thu Tran1, Reneta Slavikova2, Lucie T

Affiliations:

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Abstract:

We present a genetic map for Xenopus tropicalis, consisting of 2886 SSLP markers. Using a bioinformatics-based strategy, we identified unique SSLPs within the X. tropicalis genome. Scaffolds from X. tropicalis genome assembly 2.0 (JGI) were scanned for SSRs; unique SSRs were then tested for amplification and polymorphisms using DNA from inbred Nigerian and Ivory Coast individuals. Thus identified, the SSLPs were genotyped against a mapping cross panel of DNA samples from 192 F2 individuals. Nearly 3000 SSLPs were genotyped, yielding a 2886-marker genetic map consisting of 10 major linkage groups between 73 – 132 cM in length, and 4 smaller linkage groups between 7-40 cM. The total effective size of the map is 1630 cM, and the average intermarker distance for each linkage group ranged from 0.27 – 0.75 cM. Fluorescence In Situ Hybridization (FISH) was carried out using probes for genes located on mapped scaffolds to assign linkage groups to chromosomes. Comparisons of this map with the X. tropicalis genome Assembly 4.1 (JGI) indicate that the map provides representation of a minimum of 62% of the X. tropicalis genome, incorporating 758 of the approximately 1300 scaffolds over 100,000 bp. The genetic map and SSLP marker database constitute an essential resource for genetic and genomic analyses in X. tropicalis

Name:
Skopalík, Josef

Poster Title:
Labelling of rabbit bone-marrow cells with radioisotope In-111 and NMR contrast agents

Authors:
Josef Skopalík, Martin Pešl, Milan Richtárik, Zenon Starčuk, Jiří Štěpán

Affiliations:
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Abstract:
Backround: It has been observed in many animal models that transplantation of bone-marrow mesenchymal cells (BMMCs) induces functional improvement of infarcted heart. Tracking of injected cells in animal studies has been based on fluorescence labelling or on genetic modifications by the introduction of genes expressing fluorochromes or metabolic enzymes, however detection of fluorescence and enzyme reactions are limited by the need to kill the animals. Radioactive labeling of BMMCs with In-111 and NMR contrast agents could be a noninvasive method for tracking of BMMCs delivered into the infarcted heart. In published literature, labeling methods with In-111 and NMR contrast agents for human, canine, pig and rat BMMCs were evaluated. Evaluation of these methods is commonly based on determining of efficiency of labelling, surviving of cells and minimum number of detectable cells. Evaluation and comparison of these type of labelling methods for rabbit BMMCs is still lacking.
Methods: Rabbit BMMCs were isolated and cultured for 1-3 weeks. (A) BMMC labbeling with 111In was based on incubating with 111In-troplone complex (5-15 min). Labelling efficiency was determined. Surviving of BMMCs during 1 week was monitored. Different number of labelled cells was placed in phantom of rabbit chest and several methods of SPECT imaging were tested. (B) Two types of iron oxide particles (Resovist or supermagnetic maghemite) were added to the BMCs culture (final concentration 100 ug/mL). Labelled BMCs were washed after several days and different number of labelled cells was placed in phantom of rabbit chest and NMR imaging was tested.
Results and conclusion: (A) The labelling efficiency of best variant was about 40%, which resulted to BMMCs displaying about 3 Bq/cell . Viability was minimaly decreased by this procedure. Cluster of 5 x 10 5 BMMCs were localised and quantified using SPECT.
(B) Magnetic labeling and deadherence of cells slightly decreased the viability of BMMCs (80% after 24h). 1 x 10 5 BMMCs were localised in heart tissue using NMRi. These two methods can be used for tracking of cells delivered into the infarcted rabbit heart.

Name:
Sojka, Ludek

Poster Title:
DNA elements of promoter regulation by transcription initiation nucleosid triphosphates in Bacillus subtilis

Authors:
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Institute of Microbiology and Institute of Molecular Genetics, Prague, Czech Republic

Abstract:
To survive, the bacterial cell must promptly adapt to changes in its environment. This adaptation is mediated by changes in gene expression. Rapid changes in gene expression can be achieved by affecting the activity of RNA polymerase (RNAP) by small molecule effectors. One such small molecule effector is the unusual nucleotide (p)ppGpp, which, unlike to Escherichia coli, affects Bacillus subtilis RNAP indirectly. The other important small molecule effector is the initiating NTP (iNTP). Here, we focus on promoter DNA determinants of RNAP sensitivity to iNTP. This regulation is well defined for rRNA promoters of the gram negative bacterium E. coli. The rules derived from this model organism, however, are not universal for all bacteria. rRNA promoters of B. subtilis, a model gram positive bacterium, display sequence features that are not compatible with promoter regulation by [iNTPs] in E. coli, and yet they are regulated by GTP, their [iNTP]. We addressed this question experimentally, using a combination of in vitro and in vivo approaches. We identified a promoter region in [iNTP] sensitive promoters from B. subtilis that is crucial for this regulation and we will discuss its consequences.

Name:
Soták, Matúš

Poster Title:
Determination of distribution of circadian rhythms along rat colonic crypts using laser capture microdissection

Authors:
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Abstract:
Introduction: Organisms living in rhythmically changing environment possess timing machine based on molecular clocks. In both the central nervous system and peripheral tissues including liver and alimentary tract, clock genes exhibit rhythmic expression. The circadian clock regulates hundreds of functions including proliferation and intestinal digestion and absorption. We have shown recently in intestinal epithelium the rhythmic expression of Na(+)/H(+) antiporter Nhe3 and the cell cycle regulator Wee1, which are suggested to be clock-controlled genes. Moreover, we have demonstrated rhythmic expression of another ion transporter Dra and gama subunit of the sodium channel ENaC. Aim: As these studies showed rhythms in scrapped mucosa from a large area of colonic epithelia, the goal of this study was therefore to elucidate whether there are differences in the distribution and phase shift of circadian clock and rhythms along the base-to-mouth axis of colonic crypts. Methods: Adult male Wistar rats were kept in light-dark regime LD 12:12. Sample collection was provided every 4 hours during 24 hours, colonic tissue was snap-frozen and histological sections were prepared using cryostat. Laser capture microdissection was used for specific tissue collection of crypt base and mouth, respectively. Total RNA was isolated and examined by quantitative real time RT-PCR. Results: We demonstrated the functional intestinal circadian clock (rhythmic expression of clock genes mRNA) in both crypt base and mouth with exactly the same phase of rhythm along the crypt axis. Furthermore we found rhythmic expression of the cell cycle regulator Wee1 mRNA in both crypt base and mouth and rhythmic expression of DRA, gama-ENaC and Nhe3 mRNA in crypt mouth. Conclusion: We determined phase-synchronized expression of clock genes and clock-controlled genes in mouth vs. base of colonic crypts. The data demonstrate for the first time the functional synchronization of clocks in particular parts of crypts.
Supported by grants from the Ministry of Health no. NS 9982-4 and the Academy of Sciences no. AV0Z50110509.

Name:
Stefkova, Katerina

Poster Title:
Hematopoiesis of mouse embryonic stem cells – the role of p38alpha kinase

Authors:
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Abstract:
Embryonic stem (ES) cells are in vitro population of pluripotent cells derived from an inner cell mass of blastocyst. They are able to maintain their self-renewal and pluripotent potential without any limitation. Thus ES cells can be used for developmental studies and in case of human ES cells, also as a source for cells and tissue therapies and replacements. The aim of our work was to study the mechanisms and effectiveness of hematopoiesis in mouse ES cells. In first step, we confirmed the occurrence of hematopoiesis in our experimental model of spontaneous differentiation of ES cell by formation of kinase in embryoid bodies. Further step was the analysis of the role of p38 hematopoiesis. P38 kinases belong to the mitogen-activated protein kinase family (MAPK). Similarly to other members of MAPK family they participate in various cellular processes that influence the regulation of cell proliferation, differentiation, and apoptosis. Recently, it has been shown that p38 inhibitors inhibit differentiation of hematopoietic progenitors. Moreover recent hypothesis suggests that p38 kinases play role in the maintenance of somatic stem cells. In kinase participates on our first approach to this idea we presume that if p38 the maintenance of the hematopoietic stem and early progenitor cells, depletion -/- when compared to kinase change the dynamics of hematopoiesis in p38 of p38 the wt p38 ES cell line. This idea is tested in presented work.
This work was supported by grant from Czech Science Foundation 301/08/0717 and 204/09/H058.

Name:
Sviridova, Ekaterina

Poster Title:
Preliminary crystallographic characterization of Iron-regulated protein FrpD from Neisseria meningitidis

Authors:
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Abstract:
Fe-regulated protein D (FrpD) is a Neisseria meningitidis outer membrane lipoprotein, which binds the secreted Repeat in toxins (RTX) protein, the Fe-regulated protein C (FrpC). FrpD is a protein highly conserved in Neisseria meningitidis strains, however has no sequence homologs in other organisms. Its function and biological role still remain unknown. This project is aimed to determine the structure of FrpD protein.
The native and Se-Met substituted variants of recombinant FrpD43-271 protein were crystallized using the sitting-drop vapor-diffusion method. Diffraction data were collected to the 2.25 Å resolution for native FrpD43-271 protein and to the 2.0 Å for Se-Met FrpD43-271 protein. The crystals of native FrpD43-271 protein belong to the hexagonal space group P62, while the crystals of Se-Met substituted FrpD43-271 protein belong to the primitive orthorhombic space group P212121. The preliminary structure of FrpD protein was determined using single anomalous diffraction (SAD) method.

Name:
Švenková, Alžbeta

Poster Title:
The delta subunit of RNA polymerase from Bacillus subtilis

Authors:
Alžbeta Švenková1, Hana Šanderová1, Veronika Motáčková2, Lukáš Žídek2, and Libor Krásný1

Affiliations:
1 Institute of Microbiology, Academy of Sciences of the Czech Republic, Prague 4, Czech Republic 2 National Centre for Biomolecular research, Faculty of Science, Masaryk University, Kotlářská 2, Brno, Czech Republic

Abstract:
RNA polymerase is a well conserved essential enzyme in bacterias. However, Gram positive bacterias contain two additional subunits. One subunit.This protein is encoded by the rpoE gene, is composed of them is the of 173 amino acids and is highly acidic. It was shown that delta regulates transcription in vitro by enhancing its specifity, inhibiting the DNA melting and formation of the open complex, but the molecular details how delta mediates these effects are unknown. It has also been suggested as being important for the virulence of Staphylococcus aureus and Streptococcus agalactiae by unknown mechanisms.
Despite the biochemical knowledge about this protein, it´s physiological role remains a mystery.To elucidate the roles of delta in the cell, we solved it´s 3D structure. Furthemore, using a combination of biochemical and genetic approaches, we identified it as important for gene expression regulation by transcription initiating nucleoside triphosphates. We will also present data demonstrating that delta is crucial for the cell fitness.
This work was supported by grants from the Grant Agency of the Ministry of Health of the Czech Republic (No. NR/9138 – 3), and the Grant Agency of the Czech Republic (No. 204/09/0583).

Name:

Vanova, Katerina

Poster Title:

THE OPPOSITE EFFECT OF ESTROGENS AND BILE ACIDS ON HEME OXYGENASE ACTIVITY IN CHOLESTASIS

Authors:

Katerina Vanova1, Miroslava Zelenkova1, Dalibor Cerny2, Libor Vitek1, Lucie Muchova1

Affiliations:

1Department of Clinical Biochemistry and Laboratory Diagnostics and 2Department of Pharmacology, 1st Faculty of Medicine, Charles University in Prague, Czech Republic

Abstract:

Introduction: Heme oxygenase (HO), the key enzyme in the protection of organism against oxidative stress, catalyzes the degradation of heme to bioactive molecules carbon monoxide (CO), iron and biliverdin. Cholestasis is a serious liver disease affecting bile formation and/or flow. The products of heme catabolic pathway might protect the liver from various pathologic insults. However, the role of HO in cholestasis remains to be elucidated. The aim of this study was to characterize the HO activity in the liver of Wistar rats with ethinylestradiol (EE)-induced cholestasis and to clarify the effect of EE and bile acids (BA) on HO activity in vitro.
Methods: Wistar female rats were treated with EE (5 mg/kg s.c.) for 5 and 18 days, control animals were administered vehicle (propanediol). HepG2 cell cultures, HepG2-rNTCP (bearing transporter for conjugated BA unlike HepG2) and primary cultures of rat hepatocytes were treated with taurocholic acid (TCA) or ursodeoxycholic acid (UDCA). UDCA is a hydrophilic BA used for treatment of cholestasis. HO activity was determined as the production of carbon monoxide. The expression of HO-1 mRNA and cholestatic markers were also determined.
Results: We found that HO activity was significantly increased after 18 days of EE treatment (130%, p

Name:

Veselá, Romana

Poster Title:

The evaluation of survival and proliferation of lymphocytes in autologous mixed lymphocyte reaction with dendritic cells. The comparison of incorporation of 3H-thymidine and differential gating method.

Authors:

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Affiliations:

(1) 1st Department of Medicine - Department of Haematology, First Faculty of Medicine, Charles University in Prague and General University Hospital in Prague; (2) The Institute of Hematology and Blood Transfusion (IHBT), Prague

Abstract:

Dendritic cells (DCs) are generally considered to be the most potent antigen-presenting cells which play the key role in T-lymphocyte proferation and induction of antitumour response. The mixed lymphocyte reaction of T-lymphocytes and DCs is one of essential instruments for immunological mechanisms studies. Conventionaly used method for determination of T-lymphocytes proliferation, incorporation of 3H-thymidine, provides only general information on survival and proliferation of T-lymphocytes. The method of flow cytometry and differential gating seems to be more suitable for quantitative and also qualitative analysis of T-lymphocyte proliferation. It is based on time limited acquisition of events and on its ditribution according to forward and side scatter values. We decided to compare these two methods and determine mutual corelation and compatibility. Nine untreated patients with chronic lymphocytic leukemia (CLL), one patient with relapsed CLL and one untreated patient with B-cell non-Hodgkin lymfoma were studied. In all cases DCs promoted the survival and proferation of both CD4 and CD8 lymphocytes but the response was much more higher in CD4 subset. Both methods retain consistent tendency of survival and proliferation of CD4/CD8 lymphocytes. However, the correlation of these methods was not convincing. Therefore, both these methods might be used for evaluation of MLR, but each of them gives specific and complementary information.

Supported by: VZ 0021620808

Name:
Veselíková, Michaela

Poster Title:
First mitochondrial protein methyltransferase in Trypanosoma

Authors:
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Institute of Parasitology, Biological center ASCR v.v.i., Ceske Budejovice

Abstract:
Methylations are recently one of the most studied epigenetic mechanisms that participate in regulation of a gene expression throughout the tree of life. In this project, we focused on the characterization of the fundamental properties concerning a putative methyltransferase MT420 in Trypanosoma brucei, the pathogenic human parasite. We used tandem affinity purification tagged MT420 to determine localization of this protein within the cell and to elucidate its potential binding partners. MT420 is a first protein methyltransferase with mitochondrial localization in T. brucei and may function as a dimer. Based on its putative homology to yeast Mtq1, MT420 probably methylates the mitochondrial release factors and thus participates in regulation of mitochondrially encoded genes expression.

Name:
Vojtek, Libor

Poster Title:
Nematobacterial complex Heterorhabditis-Photorhabdus in immunity studies

Authors:
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Abstract:
Entomopathogenic nematodes are obligate insect parasites with the increasing importance as biological control agent. Furthermore, nematodes can be used as unique natural model for infection of numerous insect species combining the simultaneous action of nematodes and their symbiotic bacteria. The bacterial symbionts are essential for successful invasion to the host causing septicaemia inside and digesting host tissues. We use common entomopathogenic nematode Heterorhabditis bacteriophora associated with symbiotic bacteria Photorhabdus luminescens which are capable to kill the insect host within 24-48 hours after infection. Especially in combination with genetically tractable Drosophila melanogaster nematobacterial complex offers ideal tool for studying the insect physiology and immunity. Symbiotic bacteria can be isolated and separately used for determination of their pathogenity to insect without nematode's influence. Photorhabdus is the only natural bioluminescence genus of soil G- bacteria, therefore it is widely applied for bioluminiscence tests. We use two non-pathogenic subspecies (laumondii and kayaii) of P. luminescens for antibacterial assays based on their bioluminescence ability (e.g. complement, myeloperoxidase and antibacterial activity determination). Except P. luminescens we use genetically modified Escherichia coli K12 that carries Photorhabdus genes for bacterial luciferase (Lux) and its substrate. Bioluminescence reaction is mediated by bacterial enzyme luciferase which catalyses the oxidation of long-chain aldehyde (substrate) and reduces flavin mononucleotide with emission of light. This emission can be directly measured by the luminometer, thus we can assumed bacterial viability.
Our research is supported by grant from Grant Agency of Czech Republic (GA206/09/P470).

Name:

Volkova, Valeriya

Poster Title:

Histopathological changes in mice organs infected with Leishmania major depend on sex

Authors:

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Abstract:

There are surprisingly few studies devoted to sex differences in immune response to parasites. Leishmaniasis affects about 12 million people worldwide with 1.5–2 million new cases each year. Understanding the environmental and genetic risk factors that determine why people with the same exposure to infection differ in susceptibility could provide important findings for therapy. Many of the problems that appear in human genetic studies can be easily avoided by using mouse models.

For analyzing the impact of the host genotype on the response to L. major we use special model - 20 CcS/Dem recombinant congenic strains (RCS). Each CcS/Dem strains contains a unique random set of about 12.5% genes from the resistant donor strain STS and 87.5% genes from the susceptible background strain BALB/c. Our tests of skin lesions, splenomegaly, hepatomegaly, parasite numbers in organs and immunological parameters in individual RCS indicated that different pathological and immunological traits are largely controlled by different sets of genes that only partly overlap, which was confirmed by genetic linkage analysis of genes controlling individual symptoms of the disease. In previous experiments we have found 21 Lmr loci controlling pathology and immunological reactions in the highly resistant strain CcS-5, the intermediate strains CcS-11, CcS-20 and the highly susceptible CcS-16 strain. Taking previous results into consideration we selected for the study males and females of twelve RCS strains and parental strains BALB/c and STS. Lymph nodes from lesions, lymph nodes iliacus lateralis, lymph nodes inguinalis superficialis, liver, spleen and skin were collected from mice after 8 weeks of L. major infection. Size of organs and histopathological parameters including parasite number, signs of necrosis and apoptosis, fibrotic changes, granuloma formations, infiltrations with lymphocytes, polymorph nuclear cells, eosinophils, macrophages, plasma cells, cell detritus, activation of germinal centres in lymph nodes and white pulp micro architecture in spleen were analyzed. During study we observed large differences in parasite numbers, the amount, size and maturity of granulomas, cells infiltrations and germinal centres activation both among strains and between sexes. It suggests the existence of genes controlling infiltrations by immune cells and their possible influence on sex differences.