11TH IMG PHD CONFERENCE 29|06

CONFERENCE ABSTRACT BOOK

START: 9:00

HASEK AUDITORIUM HALL

IMG

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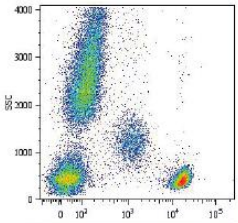
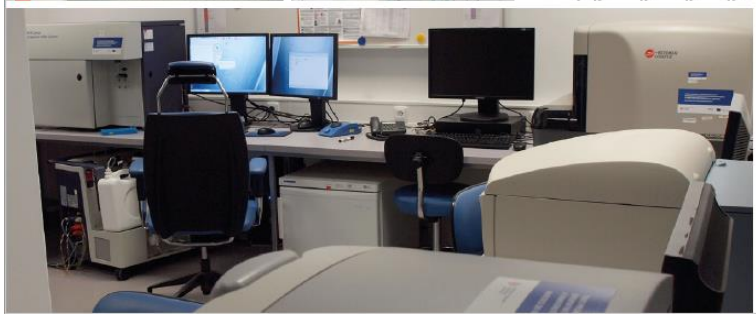
ARIB project has received funding from the European Union's Horizon 2020 programme under grant agreement No 763823





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PROGRAM

9:00 – 9:10	**Opening**
9:10 – 9:25	**Daniela Polatová** The Role of CMTM in Regulation of IL-17-Receptor Signaling
9:25 – 9:40	**Martin Kovář** Mechanisms Regulating Haematopoiesis in Cyclostoma
9:40 – 9:55	**Imtissal Krayem** Genetic Influence on Frequencies of Blood Cell Subpopulations in Mouse
9:55 – 10:10	**Zuzana Krchňáková** Polypyrimidine Tract Sequence Determines Splicing Efficiencies of lncRNAs
10:10 – 10:30	Coffee break
10:30 – 10:45	**Radka Štorchová** The Role of Wip1 Phosphatase on Chromatin
10:45 – 11:00	**Olga Babošová** The Role of EGLN2/Proline Hydroxylase 1 in Regulation of Cyclin D1 in Mantle Cell Lymphoma
11:00 – 11:15	**David Přikryl** Osteopetrosis: Somewhere Between Chronic and Acute Oncogenicity
11:15 – 12:05	**Synthetic Mammalian Gene Circuits: From Fundamentals to Application** **Dr. Yaakov Benenson**
12:05 – 13:00	Lunch

13:00 – 13:15	**Vladimír Mučejovský** Gene Flow Between Oak Species in Slovakia Analyzed by Microsatellites
13:15 – 13:30	**Matej Fabišik** Altered Immune Responses in Mice With LST1 Adaptor Protein Deficiency
13:30 – 13:45	**James Cleland** Heads or Tails: How Do Planarian Flatworms Decide What to Regenerate?
13:45 – 14:35	**Figure Design Workshop** **Dr. Helena Jambor**
14:35 – 15:00	**ARIB Networking session with a coffee** (IMG & MPI-CBG students, supported by ARIB)
15:00 – 15:50	**Deconstruction of an Enhancer Cluster in Early Embryonic Development** **Dr. Christa Bücker**
15:50 – 16:05	**Tomáš Brabec** IL-17 drives Paneth Cell-Mediated Control of Segmented Filamentous Bacteria
16:05 – 16:20	**Daniela Glatzová** Palmitoylation Determines Nanoscale Organisation of CD4 at the Plasma Membrane of T cells
16:20 – 16:35	**Davide Basello** Molecular Principles of Cajal Body Formation
16:35 – 16:50	**Katarzyna Szczerkowska** Zfp644 - Its Role on a Vision and Beyond
17:00 – 18:30	**Drinks & Poster Session**
18:40 – 18:50	**Best talk and poster award, closing remarks**
18:50 – 0:00	**Social event**

ARIB

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ABSTRACTS - TALKS

Synthetic Mammalian Gene Circuits: From Fundamentals to Application

Yaakov Benenson, PhD

Synthetic Biology Group, ETH Zürich

Synthetic gene circuits that 'compute' in situ with endogenous molecular inputs have the potential to control and reprogram cell behaviour in complex fashion. Focusing on mammalian cells, our group develops biological computing circuits that are tightly integrated with their host cells via endogenous inputs and outputs. In the talk I will describe some of the mammalian circuit design platforms we have established, such as combinatorial logic with microRNA and transcription factor inputs in order to target specific cell states. I will also introduce the concept of dynamic control of circuit genetic encoding and show how this idea can lead to dramatic consequences for circuit operation: from orders-of-magnitude improvement in sensor dynamic range, to the possibility of compressing circuit genetic encoding in a manner similar to 'zipping' a computer file. Lastly, I will describe some of the open questions and challenges that still need to be addressed before such circuits can gain a widespread acceptance and translated into (medical) practice.

Synthetic Mammalian Gene Circuits: From Fundamentals to Application

Christa Bücker, PhD

Max F. Perutz Laboratories, University of Vienna

During development, cells undergo constant cell fate transitions and each one is characterized by changes in gene expression patterns. These changes are orchestrated by cis-regulatory elements such as enhancers, short stretches of DNA sequences comprising multiple TF binding sites that drive the expression of a target gene from a distance. Many genes are regulated simultaneously by multiple enhancers, sometimes called super enhancers (SEs), in the same cell. How single elements work together to ensure the correct spatio-temporal regulation of the target gene is so far unclear. We have identified an enhancer cluster consisting of 5 individual elements that is established during the exit from naïve pluripotency. Through genome engineering and fine-tuned time courses, we have dissected the contribution of each single elements to the correct spatio-temporal expression of the target gene, Fgf5. Each element contributes in varying extend to the full expression pattern of Fgf5, with one element standing out as the main organizer of target gene expression.

The Role of CMTM in Regulation of IL-17-Receptor Signalling

Daniela Polatová[1], *Helena Draberova*[1], *Ondrej Stepanek*[1], *Peter Draber*[1]

[1]Laboratory of Adaptive Immunity, Institute of Molecular Genetics AS CR, Czech Republic

IL-17 is a crucial regulator of inflammatory immune reaction. Binding of this cytokine to its specific receptor triggers several signaling pathways resulting in the production of pro-inflammatory cytokines such as IL-6, CCL2 or CXCL1 which then recruit macrophages and neutrophils to the site of inflammation to promote an immune defense of the organism. The absence of IL-17 signaling leads to increased sensitivity to some pathogens, e.g. *Candida albicans*. If the signalization via IL-17 receptor signaling complex (IL-17-RSC) is disrupted, severe autoimmune disorders such as psoriasis, rheumatoid arthritis or multiple sclerosis occur. Currently, several antibodies against IL-17 or its receptor are in clinical use to treat psoriasis and psoriatic arthritis. In our laboratory a unique approach to study the composition of the IL-17-RSC via mass spectrometry was previously developed. The data obtained by this method repeatedly show the presence of transmembrane protein CMTM, a new and hitherto unknown component of IL-17-RSC. Here we aimed to understand the role of CMTM in the regulation of the IL-17 receptor-triggered signaling pathways and to elucidate whether the ablation of this protein could be used to modulate the IL-17-induced cellular responses.

Mechanisms Regulating Haematopoiesis in Cyclostoma

Martin Kovář, Petr Bartůněk

Department of Cell Differentiation, Institute of Molecular Genetics AS CR, Prague, Czech Republic

Haematopoiesis is process during which haematopoietic stem cells (HSC) give rise to all terminally differentiated blood cells. In all higher vertebrates from fishes to mammals, haematopoiesis share similar genetics and molecular pathways for HSC maintenance and differentiation of all blood cell lines. Our data suggest, that this is common for all vertebrates, even for one of the oldest living branches of vertebrates, the jawless. We looked for homologues of important hematopoietic genes known from higher vertebrates in transcriptomics data from the sea lamprey (*Petromyzon marinus*). We found lot of sequences highly similar to known hematopoietic genes, which play as key role in differentiation of every single blood cell lineages in higher vertebrates. Therefore we propose that the basic mechanisms of haematopoiesis are well conserved in all living vertebrates and all blood cell lineages were established early in the vertebrate evolution and by comparing differences in development and regulation of haematopoiesis we can elucidate evolution of haematopoiesis.

Genetic Influence on Frequencies of Blood Cell Subpopulations in Mouse

Imtissal Krayem[1], Yahya Sohrabi[1], Eliška Javorková[2,3], Valeriya Volkova[1], Hynek Strnad[4], Aigerim Aidarova[1], Jarmila Vojtíšková[1], Vladimír Holáň[2,3], Peter Demant[5], Marie Lipoldová[1]

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Inborn differences among individuals in frequencies of blood cell subpopulations might influence outcome of many acute and chronic conditions such as susceptibility to infections, cardiovascular diseases and cancer. We have analyzed percentage of cells subpopulations in the spleens of mouse strains O20, C57BL/10 and B10.O20 using flow cytometry. We observed higher frequency of T cell lineage cells and lower numbers of myeloid derived cells in O20 in comparison with C57BL/10. The strain B10.O20, carrying 3.6% of genes of the O20 strain on C57BL/10 background, had lower frequency of T cell subpopulations and higher frequency of myeloid derived cells than both parents. To determine the location of O20 gene(s) responsible for differences in blood cells frequencies in B10.O20, we analyzed cell frequencies in spleens of F_2 hybrids between C57BL/10 and B10.O20. B10.O20 carries O20-derived segments on four chromosomes. They were genotyped in the F_2 hybrid mice and we tested their linkage with cell subpopulations frequencies by analysis of variance (ANOVA). We have sequenced genomes of C57BL/10 and O20 and performed bioinformatics analysis of the chromosomal segments exhibiting linkage with frequencies of blood cell subpopulations. Linkage analysis revealed three novel loci. Locus on chromosome 1 control numbers of eosinophils ($CD11^+Gr1^-Siglec\text{-}F^+$), whereas locus on chromosome 18 influences frequencies of $CD19^+$ and $CD19^+CD22^+$ cells. Interaction of loci on chromosomes 1 and 17 regulates frequency of $CD11bSiglec^{hi}$ subpopulation. Analysis of these loci for polymorphisms between O20 and C57BL/10 that change RNA stability and genes' functions led to detection of 36 potential candidate genes, 2 of them carrying a non-sense mutation in the O20 strain. These genes will be focus of future studies not only in mice but also in humans.

Polypyrimidine Tract Sequence Determines Splicing Efficiencies of Long Non-Coding RNAs

Zuzana Krchňáková[1], Prasoon Kumar Thakur[1], Michaela Krausová[1], Nicole Bieberstein[1], Michaela Müller-McNicoll[2] and David Staněk[1,]*

[1]Institute of Molecular Genetics, Czech Academy of Sciences, Prague, Czech Republic
[2]Institute for Cell Biology and Neuroscience, Goethe University, Frankfurt am Main, Germany

Many nascent long non-coding RNAs (lncRNAs) undergo the same maturation steps as pre-mRNAs of protein-coding genes (PCGs) including capping and polyadenylation, but they are often poorly spliced. To identify the underlying mechanisms for this phenomenon, we used the lncRNA-a2 as model and showed that intronic sequences are primarily responsible for its inefficient splicing. Genome-wide analysis of intron splicing in intergenic lncRNAs (lincRNAs) using RNA-Seq data from five different human cell lines revealed that introns of efficiently spliced lincRNAs contain a higher thymidine content in the polypyrimidine tract (PPT) than efficiently spliced introns of PCGs. To test this effect experimentally, we raised the thymidine content in PPTs of six lincRNAs including lncRNA-a2 and observed enhanced splicing along with improved binding of the splicing factor U2AF2. Using iCLIP, we further found that lincRNA exons exhibit poor binding of the splicing enhancer proteins SRSF2, SRSF5 and SRSF6 compared to expression-matched PCG exons. We propose that lincRNAs lack the cooperative network of interactions that enhance splicing, which renders their splicing outcome more dependent on the optimality of PPT sequences and U2AF2 binding.

The Role of WIP1 Phosphatase on Chromatin

Radka Štorchová, Kamila Burdová, Libor Macůrek

Institute of Molecular Genetics AV CR, v. v. i.

Cells are constantly exposed to diverse factors that cause DNA damage, which may lead to development of genome instability and tumor generation. To avoid these deleterious consequences after DNA damage, cells activate the signalling pathway DDR (DNA damage response), in which p53 is an integral component. Wip1 phosphatase (also called PPM1D) directly dephosphorylates p53 and terminates DDR. It is still not known by which mechanism Wip1 controls p53-dependent transcription directly at promoters and what are Wip1 p53-independent functions.

In this study we focus on the role of Wip1 on chromatin since we have found that Wip1 is mostly chromatin bound and that it binds histones, especially histone H3.1 and H3.3. We have also discovered which region of Wip1 is responsible for this interaction. We have detected that Wip1 interacts with H3.3 histone chaperone DAXX which can be also dephosphorylated by Wip1. Using proximity biotinylation assay and mass spectrometry we identified potential interactors of Wip1 and found evidence that Wip1 may be associated with telomeres. Potential roles of Wip1 interaction with histones, histone chaperone DAXX and telomeres will be discussed.

The Role of EGLN2/Proline Hydroxylase 1 in Regulation of Cyclin D1 in Mantle Cell Lymphoma

Olga Babosova[1], Lucie Lanikova[1,2,3], Katarina Kapralova[2,3], Vladimir Divoky[2], Vladimir Korinek[1], Josef T. Prchal[3,4]

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Mantle cell lymphoma (MCL) is an incurable B-cell non-Hodgkin lymphoma characterized by an overexpression of cyclin D1 (CD1, encoded by the *CCND1* gene). We hypothesized that iron chelation, the known target of which is CD1, would be particularly effective in MCL. We treated human MCL cell lines with iron chelator deferoxamine mesylate (DFO), which caused decreased cell growth, increased apoptosis, and decreased CD1 mRNA and protein level. A possible cytotoxic effect due to a high concentration of DFO was ruled out by abrogating the DFO effect by concomitant administration of ferric ammonium citrate. Expression analysis of several hypoxia related genes in DFO treated MCL cell lines revealed down-regulation of prolyl hydroxylase PHD1. To further confirm that *EGLN2*/PHD1 inhibition is linked to suppression of CD1, we treated MCL cell lines with PHD inhibitors DMOG and FG-4497. Proliferation was markedly reduced and down-regulation of CD1 was confirmed in mRNA and protein levels. It has been proposed that the *EGLN*2/PHD1 substrate linked to repression of CD1 is the transcription factor FOXO3. We measured FOXO3 expression levels in MCL cell lines after treatment with DFO and DMOG and found it significantly upregulated. However, knock-out of *FOXO3* by CRISPR/Cas9 technology in MCL line Mino did not affect CD1 levels after DFO treatment. These data suggest that in MCL cell lines treated with DFO, accumulation of FOXO3 is present, but not required for CD1 repression and that CD1 down-regulation mediated by inhibition of PHD1 is a result of another, as yet unknown mechanism.

Osteopetrosis: Somewhere Between Chronic and Acute Oncogenicity

David Přikryl, Vladimír Pečenka, Vít Karafiát, Dana Kučerová, Josef Geryk, Daniel Elleder, Jiří Hejnar

Department of Viral and Cellular Biology, Institute of Molecular Genetics, Prague,

Avian leukosis viruses (ALVs) are simple retroviruses from Alpharetrovirus genus that cause various diseases in G*alliformes*. In the past, several strains of ASLV with exceptionally strong pathogenicity have been described (Kirev et al., 1989). The most common ALV-associated pathologies are the monoclonal or oligoclonal tumours/leukemias induced via insertional mutagenesis and transactivation of adjacent genes. In contrast, osteopetrosis, a disease characterized by extremely hyperplastic long bones and increased osteoblast proliferation is polyclonal and the mechanism of its induction remains obscure (Aurigemma et al., 1989, 1990, 1991).

Among the ALVs, the myeloblast-associated virus 2 (MAV-2) derives an acute osteopetrotic variant MAV-2(O). We inoculated intravenously chicken embryos with MAV-2(O) and correlated time of infection, dose of the virus and route of infection with latency and severity of osteopetrosis. Similarly to diseases caused by acutely oncogenic avian sarcoma viruses, early time of infection and high virus dose markedly increased the severity and reduced the latency of osteopetrosis. Injection of virus-producing cells instead of virions did not promoted osteopetrosis (as opposed to lung and liver tumors induced by MAV-2); this mode of infection rather reduced disease severity.

Comparison of several osteopetrotic and non-osteopetrotic MAV-2 variants (as well as several other ALVs) revealed a few single nucleotide differences that strongly correlated with osteopetrotic potential; they were situated in LTR, integrase and transmembrane region of env.

Future experiments are designed to pinpoint and verify which of these polymorphisms are crucial for the induction of osteopetrosis, how they affect functions of the respective parts of the virus genome and eventually how it all can stimulate proliferation of osteoblasts.

Gene Flow Between Oak Species in Slovakia Analyzed by Microsatellites

Vladimir Macejovsky

Faculty of Forestry, Department of Phytology, Technical University in Zvolen

The *Quercus* species are well known for their complicated and controversial systematics, which is the result of wide morphological variability appearing even within the species and the frequent interspecific hybridization. The hybridizations lead to the gene flow between the species by the fully functional hybrids, which is the main reason of sharing a substantial part of the nuclear genome among the species. This sharing leads to a low genetic differentiation between oak species, which can culminate to doubts about their taxonomic status. For identifying the hybrid populations and their parental species and also estimating the extent of the shared nuclear genome among species, we have sampled seven *Quercus robur*, seven *Quercus petraea* and five *Quercus pubescens* populations from Slovakia. By using two multiplexes of primers, which showed in earlier studies high delimitation effect, we have obtained the targeted segment of DNA from individuals from these populations, which were subsequently genotyped and then analyzed by Bayesian clustering method. From the results, we have clearly identified two *Q. pubescens* and *Q. petraea* hybrid populations and one *Q. robur* and *Q. pubescens* hybrid population. Even so, the Bayesian clustering method has identified species and populations clearly, the gene flow (Nm=3.767) is still high and genetic differentiation is still low among populations.

Altered Immune Responses in Mice With LST1 Adaptor Protein Deficiency

Matej Fabisik[1], Jarmila Kralova[1], Jolana Tureckova[2], Simon Borna[1], Tereza Skopcova[1], Jan Prochazka[2], Frantisek Spoutil[2], Bernard Malissen[3], Radek Sedlacek[2], Tomas Brdicka[1]

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Transmembrane adaptor protein LST1 is expressed in leukocytes of the myeloid lineage. Previous study has revealed mild effects of LST1 deficiency on the outcome of influenza infection in mice. Except for this specific case, its overall function at the organismal level is still to be determined. At the molecular level, LST1 was shown to interact with cytoskeleton regulating proteins and to promote the formation of tunneling nanotubes. It also contains an ITIM motif in its intracellular tail, which was shown to bind phosphatases SHP-1/2 in monocytes. To study the physiological function of LST1 we have performed a thorough analysis of LST1-deficient mice. At steady state, these mice displayed no apparent phenotype. However, when we challenged LST1-deficient mice with pro-inflammatory stimuli some aspects of their responses were altered. IP injection of viral mimetic PolyI:C resulted in significant reduction in splenic $CD8^+$ T cell percentages. However, the most striking differences were observed when we induced acute colitis in these mice by dextran sodium sulphate, as a model of disease, where myeloid cells are heavily involved. We found significantly better course of acute colitis in LST1-deficient animals in all observed parameters (body weight, colon length…). This was accompanied by alterations in splenic monocyte populations. Interestingly, we also saw the same significant decrease in CD8+ splenic T cells as after polyI:C injection. Collectively our data suggest, that LST1 is not required for leukocyte development and immune system homeostasis, but it is involved in the regulation of several types of immune responses.

Heads or Tails: How Do Planarian Flatworms Decide What to Regenerate?

Cleland JP, Vu HTK & Rink JC

Max Planck Institute of Molecular Cell Biology and Genetics, Pfotenhauerstrasse 108, 01307 Dresden, Germany

Although we humans have very limited regenerative powers, many animals have the extraordinary ability to replace any missing body part. One critical but poorly understood aspect of this phenomenon is how regenerating wounds “decide” what body part to make. We are attempting to address this question in the undisputed champion of regeneration, the planarian flatworm. If one of these animals is cut into tiny pieces, each of the pieces will regenerate a head at the front and tail at the back. We and others have shown that a Wnt signalling “switch” determines whether head or tail will be regenerated, but how the switch is flipped on/off according to whether the wound is at the front or the back of a piece is not well understood. Classical experiments suggest that some kind of “polarity” along the planarian head-tail axis controls the Wnt switch, but its molecular nature is not known. I will present our ongoing efforts to identify the polarity cue/s by complementary unbiased and candidate-gene approaches.

IL-17 Drives Paneth Cell-Mediated Control of Segmented Filamentous Bacteria

Tomáš Brabec, Matouš Vobořil, Adéla Čepková, Martin Jakubec, Dominik Filipp

Institute of Molecular Genetics of the ASCR, v. v. i., Prague, Czech Republic

Paneth cells (PCs) are critical players in a complex network that maintains the homeostasis of intestinal immune system. Their major functions are protection from intestinal pathogens and shaping the of the intestinal microbiota composition. Effector molecules responsible for these functions are enteric α-defensins and other antimicrobial products. Their proper production and secretion is a tightly controlled process. Helper T cell (Th) responses type 1 and type 2 were both shown participate in this process, however there is so far very limited information of Th17 involvement in this process. Recently it was shown that IL-17 receptor (IL-17R) signaling in the intestinal epithelium controls the expression of EDs. This mechanism seems to be crucial to limit the number of segmented filamentous bacteria (SFB), intestinal commensal strain with the profound capability to induce Th17 responses. However, from previous studies it is unclear if IL-17 acts directly on PCs. Therefore, we analyzed the expression pattern of IL-17R in the small intestine epithelium by flow cytometry and found that PCs are the major population expressing this receptor. We also confirmed that this receptor is functional *in vitro*. Notably, injection of IL-17 into mice resulted in the increase of EDs expression in the ileum. Conversely, IL17 administered mice had significantly lower number of SFB in their intestine. Together, our results reveal a new mechanism regulating intestinal homeostasis via IL17 mediated stimulation of Paneth cells, which in turn regulate SFB numbers.

Palmitoylation Determines Nanoscale Organisation of CD4 at the Plasma Membrane of T Cells

Daniela Glatzová[1, 2], Tomáš Lukeš[3], Christian Franke[4], Zuzana Kvíčalová[1], Tomáš Chum[1], Theo Lasser[3], Sebastian van de Linde[5], Tomáš Brdička[2], & Marek Cebecauer[1]

[1] J. Heyrovsky Institute of Physical Chemistry and [2] Institute of Molecular Genetics, Academy of Sciences, Prague, Czech Republic

[3] EPFL Lausanne, Switzerland, [4] MPI Dresden, [5] University of Strathclyde, Glasgow

The surface glycoprotein CD4 is essential for maturation of T lymphocytes in thymus and is involved in their activation in peripheral tissues. CD4 enhances T cell responses to suboptimal antigens by stabilising TCR-MHC complex but was also suggested to function as an adhesive molecule. We were interested about its nanoscopic localisation on the surface of T cells. Our recently developed quantitative cluster analysis based on SOFI super-resolution imaging demonstrates an intricate nanometer organization of CD4 on resting T cells. The clustering depends on the intact extracellular domain and palmitoylation sites of CD4, since mutants lacking these structural elements exhibit random distribution. To further elucidate CD4 clusters in three-dimensional (3D) space, we developed an advanced version of the recently reported photometric 3D super-resolution method called temporal, radial-aperture-based intensity estimation (TRABI). Utilising a biplane detection scheme and directly measured single-molecule emitter intensities from both axial channels, we were able to map protein distribution on ruffled plasma membrane with 3D nanometer resolution. We demonstrate that native CD4 accumulates in high density regions which represent 'tips' in the nano-topology of membrane ruffles and microvilli. A mutant of CD4 which cannot be post-translationally palmitoylated randomly covers the ruffled surface of resting T cells. Altogether, our data suggests that, similar to TCR, monomeric CD4 accumulates on microvilli of resting T lymphocytes, a process which depends on palmitoylation of this co-receptor.

Molecular Principles of Cajal Body Formation

Davide A. Basello[1], Ivan Novotny[1], Michaela Efenberkova[1], Ales Benda[2], David Stanek[1]

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[2]BIOCEV, Vestec, Czech Republic

The cell nucleus is a highly heterogeneous environment overcrowded of molecules. Part of the nuclear complexity rises from the presence of a number of different bodies, non-membrane bound structures, which accumulate various proteins and, often RNAs. The molecular principles behind bodies assembly and maintenance are recently a matter of an intensive debate. One of the "classical" examples of a nuclear body is the Cajal body (CB). CBs are involved in biogenesis, quality control and recycling of spliceosomal snRNPs. Coilin, the essential scaffolding protein of CBs, self-oligomerize and interacts with numerous proteins including snRNPs, and these interactions are important for CB formation. However, the basic information regarding its structure and function are lacking. Here, we test whether and how the interaction of snRNPs with coilin affects coilin self-oligomerization and CB formation. In our study we combine different live cell microscopy techniques together with molecular biology and biochemistry data in order to model CB assembly, maintenance and function.

Zfp644 - Its Role on a Vision and Beyond.

Katarzyna I. Szczerkowska, Silvia Petrezselyova, Jiri Lindovsky, Marcela Palkova, Jan Dvorak, Peter Makovicky, Agnieszka Kubik – Zahorodna, Bjoern Schuster, Inken M. Beck, Jan Prochazka, Radislav Sedlacek

Academy of Science of Czech Republic, Institute of Molecular Genetics, Laboratory of Transgenic Models of Diseases

ZNF644 is a C2H2 zinc finger gene encoding a putative transcription regulator, of which a point mutation (S672G) is associated with inherited high myopia in humans. We applied TALEN technology to generate mutant mice either with the disease-carrying mutation (Zfp644S673G) or truncating form of the Zfp644 protein (Zfp644Δ8) to reveal its impact on a vison. Both models were analyzed, in particular for the vision function and eye morphology as well as for fertility, cardiology, body composition and more. We found out that both mutant lines mimic myopia phenotype in humans. Nevertheless, additional phenotyping examinations revealed semi-fertility in Zfp644Δ8 females. We ran several breeding experiments, supported by ultrasound examination, and estrus cycle analysis that confirmed our findings. However, no significant difference in ovarian morphometry was found. We applied ovarian transplantation as a rescue experiment. It showed that Zfp644Δ8 homozygotes ovaries are fully functional in WT organism. Our findings indicate, that semi-fertility in Zfp644Δ8 females is not a result of ovary alternation, but a whole organism homeostasis dysfunction. Further experiments are needed to reveal the molecular mechanism besides it.

ABSTRACTS - POSTERS

1 - Toll-like receptor 2 expressing erythro-myeloid progenitors are critical for early embryonic development

Iva Splichalova[1,2], Jana Balounova[1], Dominik Filipp[1]

[1]Department of Immunobiology, Inst of Molecular Genetics AS CR, Prague, Czech Republic
[2]Department of Cell Biology, Charles University in Prague, Czech Republic

Mammalian hematopoiesis is established during early embryonic development. Toll like receptors (TLRs) are critical for pathogen recognition and regulation of innate and adaptive immune responses. Here, we show that TLR2 is expressed on the surface of erythro-myeloid progenitors (EMPs) which colonize embryo. Additionally we demonstrated the importance of TLR2+ EMPs in early embryonic development by specific depletion of TLR2+ cells.

2 - U6-specific factor SART3 participates in biogenesis of Sm-class snRNPs

Klara Klimesova[1] and David Stanek[1]

[1]Department of RNA Biology, Institute of Molecular Genetics CAS, 142 20 Prague, Czech Republic

Spliceosomal small nuclear RNPs are assembled in a multi-step biogenesis pathway. A final assembly stage occurs in Cajal bodies and protein SART3 is important for the proper localization of snRNPs into Cajal bodies. However, the molecular mechanism remains unclear. Here we show that SART3 interacts with Sm proteins of immature snRNPs.

3 - Pseudohyphal formation in wild strains of *Saccharomyces cerevisiae*

Pavla Novotná[1,2], Martin Kuthan[1], Libuše Váchová[2] and Zdena Palková[1]

[1] Department of Genetics and Microbiology, Charles University, BIOCEV, Czech Republic, novotpa@natur.cuni.cz
[2] Institute of Microbiology AS CR, Prague, BIOCEV, Czech Republic

Pathogenic yeasts are able to make our life unpleasant by their ability to inhabit our tissues. Yeasts use special form of cells called pseudohyphae for this ability. The brewer´s yeast *Saccharomyces cerevisiae*, is able to switch to filamental growth and serves therefore as a model of invasivity of pathogenic yeasts.

4 - 'Bystander' effect of tumour cells induced to premature senescence

O. Sapega[1], R. Mikyšková[1], B. Mrázková[2], Hodný Zdeněk[2], M. Reiniš[1]

[1]Laboratory of Immunological and Tumour Models, Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czech Republic
[2]Laboratory of Genome Integrity, Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czech Republic

Distinct agents can display different effects on tumour cells in terms of senescence induction. We have demonstrated that docetaxel induced deep senescence both in the TC-1 and B16 murine tumour cell lines. On the other hand, treatment with IFNγ+TNFα induced a proliferation arrest only in B16 cells.

5 - BRAT1 - not only a spoiled child

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BRAT1 was recently identified as BRCA1-associated ATM activator-1. *We* aim to *examine* two patients with the unpublished mutation in BRAT1 gene exhibited neuropathology. However, besides depletion of BRAT1 protein, patients don't have any problem during DNA damage repair. The exact role of BRAT1 protein hasn't yet been revealed and we have a great opportunity to contribute to its identification.

6 - The role of cullin-RING ubiquitin ligases in orofacial development

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A large body of evidence implicates a role of cullin-RING ubiquitin ligases (CRLs) in cancerogenesis but very little is known about their physiological functions during development. Our experiments bring first evidence that the CRLs play crucial role in tooth formation during embryogenesis and impact important molecular pathways involved.

7 - The Role of Phosphoinositides in Chromatin Remodeling

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Phosphoinositides (PIs), small, negatively charged phospholipids, have been shown to play key roles in several physiological and pathological processes. Several studies have reported the involvement of phosphoinositides in chromatin remodeling. In this study, we are focusing on two chromatin-associated proteins - histone deacetylase-1 (HDAC-1) and barrier-to-autointegration factor (BAF), and their possible association with phosphoinositides.

8 - The regulatory role of CLR4 complex in homeostasis and cancer development in the gastro intestinal tract

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CRL4 is important ubiquitin ligase complex, responsible for regulation of signaling pathways. Cul4a is expressed in crypt base population and knockout leads to misshapen villus morphology, corresponding to enlarged proliferative zone. Combination of Apcmin and Cul4KO leads to increased severity of tumor formation, suggesting regulatory role of Cul4a in cancer progression.

9 - Role of *Cnot6l* in maternal mRNA turnover

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The oocyte-to-embryo transition (OET) is one of the most dramatic transitions in biology - highly differentiated oocyte undergoes numerous coordinated changes and is converted into totipotent blastomeres. In mouse, CCR4-NOT complex with its deadenylase component CNOT6L plays important role in crucial maternal mRNA degradation process during OET.

10 - Can Dicer boost antiviral immune response in mammals?

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We generated mice with highly active Dicer isoform – DicerO and we want to test if these mice would have enhanced antiviral RNA interference (RNAi). RNAi is used as an antiviral immune response in plants and invertebrates but has been replaced by IFN response in mammals, thus we want to revive this ancient antiviral mechanism in mammals.

11 - Vinculin is required for meiotic progression in mouse spermatogenesis

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The focal adhesion protein vinculin has various functions in the cytoplasm; however, the nuclear role of VCL remains completely unknown. Here we indicate for the first time that VCL does not act only in the cytoplasm, but it is also involved in events occurring in the meiotic nuclei during mouse spermatogenesis.

12 - Protein tyrosine phosphatase SHP-1 modulates microtubule nucleation in mast cells

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The mechanisms underlying microtubule reorganization are largely unknown. Here we report on the regulation of microtubule nucleation in bone marrow-derived mast cells by SHP-1 protein tyrosine phosphatase. We propose that SHP-1 phosphatase represents a new negative regulator of microtubule nucleation in activated mast cells.

13 - Deletion of mouse Fmr1nb gene leads to errors in chromosome alignment and male subfertility

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To err is human, but errors during germ cell differentiation often lead to infertility. Mammalian meiosis requires many protein factors already discovered, however some remain unidentified. We have generated null mutant of Fmr1nb gene, which shows testis specific expression, to address its role in the course of mouse spermatogenesis.

14 - Bioinformatics Analysis of Splicing Efficiency in Long Non-Coding RNAs

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Long non-coding RNAs (lncRNAs) are less efficiently spliced than protein coding genes (PCG). Herein, we have performed the genome wide bioinformatics analysis of long intergenic non-coding RNAs (lincRNAs) to understand the possible reasons of less-efficiently splicing of lincRNAs then.

15 - Toll-like receptor signaling in thymic epithelial cells increases cooperative antigen transfer an enforces the induction of immune tolerance

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Cooperative antigen transfer (CAT) from medullary thymic epithelial cells (mTECs) and thymic dendritic cells is essential for induction of immune tolerance. We show, that Toll-like receptor signaling in mTECs increases CAT and enforces the induction of immune tolerance by increase production of regulatory T-cells.

16 - Abrogation of interferon gamma (IFN-γ) signalling does not necessarily worsen sensitivity to PD-1/PD-L1 blockade

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Programmed death-ligand 1 (PD-L1) blockade is a novel approach for cancer treatment. However, response rate is at most 30%. Therefore, predictive markers are necessary. The aim of our study was to assess if a non-functional interferon gamma signalling in tumours with PD-L1 and MHC-I irreversible downregulation interferes with anti-PD-L1 cancer therapy.

17 - The Sm-core mediates the retention of partially-assembled spliceosomal snRNPs in Cajal bodies until their full maturation

Adriana Roithová[1], Klára Klimešová[1], Josef Pánek[3], Cindy L. Will[2], Reinhard Lührmann[2], David Staněk[1] and Cyrille Girard[2]

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snRNPs are essential components of the spliceosome. Their biogenesis starts by transcription and ends in the Cajal bodies, where the specific proteins are bound and final quality control step is provided. However, the mechanism of their final maturation is still unknown. Here we propose a model how are the immature snRNPs navigated and controlled in the CBs.

18 - *Prdm9*-controlled meiotic chromosome interactions in hybrids between closely related mouse subspecies

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Hybrid sterility is one of the reproduction isolation mechanisms leading to speciation. The broadly accepted view is that the sterility is caused by interactions of high number of genes. Using our mouse model we uncovered a non-genic, chromosomal component of incompatibility, which is controlled by the *Prdm9* gene.

19 - Chronic inflammation affects hematopoietic stem cells

Srdjan Grusanovic[1], Petr Danek[1], Miroslava Kardosova[1], Monika Burocziova[1], Jarmila Kralova[2], Tomas Brdicka[2], and Meritxell Alberich-Jorda[1]

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Hi! I'm Srdjan and I work on hematopoietic stem cells. When my mice are sick, their hematopoietic stem cells are sick as well, but at some point they behave like athletes of steroids. Come to see my poster and I will introduce myself, my mice and their inflamed hematopoietic stem cells.

20 - A Missense Mutation in Prp16 That Causes Retinitis Pigmentosa

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A large number of human diseases are consequence of splicing errors but some show a tissue specific phenotype although they are caused by mutations in ubiquitously expressed spliceosome components. Retinitis pigmentosa (RP) fits nicely in this category and missense mutation in splicing protein PRP16 was associated with RP. Our preliminary data are showing that PRP16 have a role in a quality control of splicing and mutated protein results in higher splicing efficiency rate and more usage of a cryptic splice site.