20th JUNE 2014

7th PhD CONFERENCE

Claus M. Azzalin:
"Telomeric noncoding RNAs: functions and dysfunctions"

Claudio Sunkel:
"The role of SAC genes in cell division: genomic stability and tumorigenesis"

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Programme

9:00-9:15	**Opening - Václav Hořejší**
9:15-9:35	Helena Fábryová: The Once and Future Virus
9:35-9:55	Barbora Antošová: How Is Pax6 - Key Regulator of Eye Development – Regulated During Lens Induction?
9:55-10:15	Ilona Kalasová: Visualization of Nuclear Phosphoinositides
10:15-10:35	Dalibor Miklík: Keep Calm and Express Yourself – The Case of the Integration Site-Dependent Retroviral Expression
10:35-11:00	**Coffee break**
11:00-12:00	**EMBO YOUNG INVESTIGATOR LECTURE** Claus M. Azzalin: Telomeric Noncoding RNAs: Functions and Dysfunctions
12:00-13:00	**Lunch**
13:00-13:20	Zuzana Nahácka: Methodical Approaches for the Detection and Quantification of Programmed Cell Death
13:20-13:40	Jana Oltová: Using Zebrafish to Decipher Disp3 Function
13:40-14:00	Adriana Roithová: U2 Targeting to Cajal Bodies
14:00-15:00	**KEYNOTE LECTURE** Claudio Sunkel: The Role of SAC Genes in Cell Division: Genomic Stability and Tumorigenesis
15:00-15:20	**Coffee break**
15:20-15:40	Jitka Stančíková: Organoid Cultures: Make Your Own Tiny Organ Model
15:40-16:00	Eliška Svobodová: Reactivation of RNA Interference in Mammals – a TALE of Dicer
16:00-16:20	Matyáš Šíma: New Genetic Model for Analysis of Susceptibility to Leishmania Major
16:20-00:00	**Voting for the Best Presentation**, Best Presentation announcement, closing remarks & party

The once and future virus

Helena Fábryová

Laboratory of Viral and Cellular Genetics, Institute of Molecular Genetics of the ASCR

Endogenous retroviruses (ERVs) originate by germline infection and subsequent mendelian inheritance of their exogenous counterparts. With notable exceptions, all mammalian ERVs are evolutionarily old and fixed in the population of its host species. As a model system, we are using an ERV in mule deer (Odocoileus hemionus), which is forming new germline insertions in the natural host population in the present time. This allows us to study virus and host characteristics that accompany genome invasion by a retroelement. We have previously defined thousands of highly polymorphic integrations of the deer ERV using next generation sequencing methods. In this presentation we will focus on virological characterization of this novel gammaretrovirus, which we named Cervid endogenous gammaretrovirus (CrERV). We have been able to induce infectious virus production by cocultivation with susceptible human cells. The virus particles show typical density upon ultracentrifugation and can be visualized by electron microscopy. Interestingly, the induced virus seems to have a xenotropic host range and possesses other characteristics that can be hypothesized to be related to its recent and efficient generation of germline copies.

How is Pax6 - key regulator of eye development – regulated during lens induction?

Barbora Antošová

Laboratory of Transcriptional Regulation, Institute of Molecular Genetics of the ASCR

Lens morphogenesis is a strictly regulated complex process and Pax6 transcription factor is considered as a master regulator of eye development. Based on biochemical data, Meis1 and Meis2 transcription factors play the crucial role in Pax6 regulation during the placodal stage of lens morphogenesis. Despite these data Meis1 or Meis2 deficient mice have surprisingly mild ocular phenotype. As Meis1 and Meis2 show similar expression pattern during placodal stage they are most likely genetically redundant. For this reason, we used mouse strain deficient for both Meis1 and Meis2 at the placodal stage and performed its detailed analysis. Deletion of both Meis1 and Meis2 at the placodal stage results in loss of Pax6 expression in lens placode and loss of lens formation. Therefore, our results show that Meis transcription factors can be considered as key regulators of Pax6 during lens induction.

Visualization of nuclear phosphoinositides.

Ilona Kalasová

Laboratory of Biology of the Cell Nucleus, Institute of Molecular Genetics of the ASCR

Phosphoinositides (PIs) are phosphorylated forms of phosphatidylinositol. They are well known cytoplasmic signalling molecules but an emerging evidence points towards their roles also in the cell nucleus. The most studied nuclear phosphoinositide is phosphatidylinositol (4,5)-bisphosphate (PI(4,5)P2) that localizes to nucleoli, nuclear speckles, and small foci in the nucleoplasm. It is required for DNA transcription, pre-mRNA processing, and export of mRNA. Since many PIs metabolizing enzymes reside within the nucleus, it is plausible that also other PIs are formed in this compartment. Indeed, biochemical approaches confirmed the existence of nuclear phosphatidylinositol 3-phosphate (PI3P), phosphatidylinositol 4-phosphate (PI4P), phosphatidylinositol 5-phosphate (PI5P), and phosphatidylinositol 3,4,5-trisphosphate (PI(3,4,5)P3). However, their exact localization within the nuclear compartments remains largely unknown. Here we present PIs-specific protein domains as tools for nuclear PIs visualization in fixed cells. We believe that the knowledge of PIs localization will shed light on a complex network of nuclear PIs and their metabolizing enzymes and will help to uncover their possible nuclear functions.

Keep Calm and Express Yourself – The Case of the Integration Site-Dependent Retroviral Expression

Dalibor Miklík

Laboratory of Viral and Celular Genetics, Institute of Molecular Genetics of the ASCR

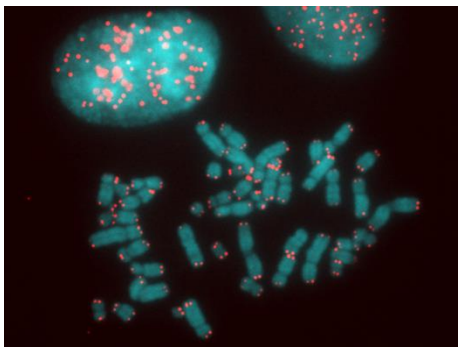
The integration of retroviral DNA into the host genome is the key step in retroviral life cycle allowing the expression of retroviral genes. However, the expression of retroviral genes is frequently silenced on transcriptional level. Since individual proviruses differ in their ability to effectively express their genes, the nature of the site of the integration is probably one of the major factors affecting transcriptional activity of the provirus. In our project, we reveal the impact of the nature of the integration site by using distinct retroviral vectors bearing green fluorescent protein as a marker of proviral activity. We generate single-provirus containing cellular clones of which long term expression profiles reflect the activity of particular proviruses. Using this approach we are able to compare the ability of retroviral vectors of different origin to establish stable expression. By searching for genomic and epigenomic features present at the integration sites we aim to identity the features defining the transcriptional, mainly active, state of the provirus expression. Such information can be useful for the design of effective retroviral vectors for transgenesis and gene therapy as well as for the treatment of silent reservoir in human immunodeficiency type-1 (HIV-1) infected patients.

Telomeric noncoding RNAs: functions and dysfunctions

Claus M. Azzalin

ETH Zürich, Institute of Biochemistry (IBC), www.bc.biol.ethz.ch/research/azzalin

The extremities of eukaryotic chromosome comprise major blocks of constitutive heterochromatin. Using mammalian cultured cells and the fission yeast *Schizosaccharomyces pombe*, we identified a number of RNA polymerase II-dependent long noncoding RNAs (lncRNAs) originating from chromosome ends. Among these transcripts, TERRA mostly contains telomeric RNA repeats and remains associated to telomeres post-transcriptionally. Our laboratory uses a combination of cellular and molecular biology, biochemistry, genetics and dynamic light microscopy to study the cellular roles and regulation of TERRA. As TERRA is refractory to commonly used down-regulation protocols, we have developed alternative tools to experimentally modulate TERRA transcription in living cells. With these tools in hands, we are dissecting TERRA contribution to fundamental biological processes such as telomerase-mediated and homologous recombination-mediated telomere length maintenance, cancer cell proliferation and cellular senescence. We are also much intrigued by the cellular pathways that prevent TERRA, and possibly other chromatin-associated lncRNAs, from harming genome integrity by interfering with DNA metabolism processes such as DNA replication, DNA repair and nucleosome positioning. In particular, given the spatial proximity of TERRA to its template DNA, we are trying to understand how cells dismantle or avert formation of potentially harmful RNA:DNA hybrid structures during telomere replication. Our research aims at expanding our current knowledge of how telomeres and other genomic regions associated to lncRNAs are maintained and exert their functions. Because of the intimate liaisons between telomere and genome integrity, cancer, cellular senescence, and organismal aging we hope to shed light on how lncRNAs impact on crucial aspects of human life.



Methodical approaches for the detection and quantification of programmed cell death

Zuzana Nahácka

Laboratory of Cell Signalling and Apoptosis, Institute of Molecular Genetics of the ASCR

Cell death as the terminal process in cell existence could be triggered by various stimuli. Prominent among them is physical, chemical or biological stress leading to structural damage or severe destabilization of essential metabolic and signaling processes, and ultimately ending in accidental or programmed/regulated destruction of affected cells. Programmed cell death plays an essential role during embryogenesis, maintenance of tissues in adult organisms and in the immune response. The default mode of programmed cell death in most of these processes is caspase-dependent apoptosis. However, in addition to apoptosis, recently regulated necrosis as well as cell death associated with autophagy turned out to be physiologically relevant as well. In the first part of my presentation I shall introduce basic principles of different types of cell death. The second part will be focused to various principles and derived methodical approaches used for the determination of cell death in cells and tissues. These methods are based on the analysis of metabolic activity, cleavage of chromosomal DNA, loss of plasma membrane integrity, cell morphology or some distinct biochemical features. I shall introduce some of the most important methods with some practical examples from my work.

Using zebrafish to decipher Disp3 function

Jana Oltová

Department of Cell Differentiation, Institute of Molecular Genetics of the ASCR

Dispatched 3 (Disp3) is a protein related to the members of Patched and Dispatched families, which are known for their substantial role in Hedgehog signaling. Several years of extensive studies provided indications of its function in cholesterol metabolism, proliferation and differentiation of neural cells. However, the function of Disp3 at the level of the whole animal still remains to be deciphered. Our main goal is to investigate whether there is any role of Disp3 in the Hedgehog signaling and to characterize it in the context of the whole pathway. Moreover, we are particularly interested in the potential function of Disp3 in the development, as we know that the onset of its expression happens rather early in the developing animal and is restricted mostly to the brain and the eye. Using zebrafish, we now have plenty of tools that can help us answer all these questions and gain new insights into Disp3 function.

U2 targeting to Cajal bodies

Adriana Roithová

Laboratory of RNA biology, Institute of Molecular Genetics of the ASCR

In the cell we can find a lot of small noncoding RNAs, which are important for many processes. Among those RNAs are small nuclear RNA uridin rich, which with proteins create U snRNP. These particles play important role in pre-mRNA splicing. In this process are noncoding sequences (introns) removed and coding sequences (exons) are joined. It is catalyzed by multiprotein complex called spliceosome. The core of the spliceosome is created by U1, U2, U4, U5 and U6 snRNP. So these particles are essential for this process. Some steps of U snRNP biogenesis proceed in nuclear structures called Cajal bodies (CB). In my thesis I focused on factors, which are important for targeting U snRNA into CB. I used U2 snRNA like a model. With the aid of microinjection of fluorescently labeled U2 snRNA mutants I found, that the Sm binding site on U2 snRNA is essential for targeting to CB. Knock down of Sm B/B´ showed us, that Sm proteins are necessary for transport U2 snRNA to CB. Sm proteins are formed on U2 snRNA by SMN complex. Deletion of SMN binding site on U2 snRNA had the same inhibition effect. From these results we can see, that Sm proteins and SMN complex are important for U2 snRNA biogenesis especially for targeting into CB.

The role of SAC genes in cell division: genomic stability and tumorigenesis

Claudio Sunkel

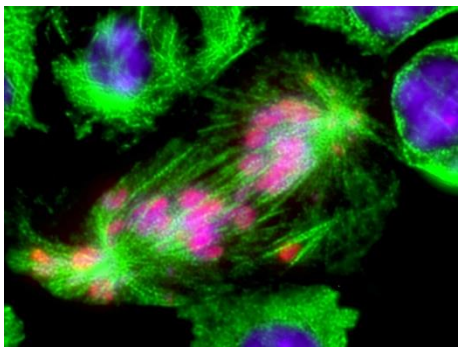
IBMC, Instituto de Biologia Molecular e Celular, Universidade do Porto

Maintenance of genomic stability during cell division relies to a large extent on the spindle assembly checkpoint (SAC). The SAC monitors kinetochore-microtubule attachment, preventing APC/C-mediated mitotic exit until proper microtubule-kinetochore attachment is achieved. In the absence of microtubule attachment genes involved in SAC signalling localize to kinetochores and generate a “wait anaphase” signal that prevents mitotic exit. Recently we have shown that a cascade of protein kinases including Polo, Aurora B, Mps1 and BubR1 play a major role in the production of an effective APC/C inhibitor. Interestingly, SAC genes also appear to be involved in other aspects of genomic stability when analysed in specific tissues within a developing organism. Down-regulating Bub3, BubR1 or Mad2 within the developing wing imaginal cells causes cell death. However, when apoptosis is prevented these mutant cells transform and effectively become tumorigenic with high levels of aneuploidy, cell autonomous growth and extensive proliferative potential. Importantly, SAC genes prevent tumorigenesis in these cells independently of their kinetochore localization suggesting that they might function as classical tumour suppressors. Our recent work in these two areas will be presented.

Conde, C., et al (2013). Polo controls Mps1-dependent BubR1 hyperphosphorylation to promote Cdc20 kinetochore recruitment and sustained Spindle Assembly Checkpoint. EMBO J. doi:10.1038/emboj.2013.109.

Morais-da-Silva, S., et al (2013). A tumour Suppressor Role of the Bub3 Spindle Assembly Checkpoint Proteins after Apoptosis inhibition. J. Cell. Biol. doi:10.1083/jcb.201210018.

Legend to the image: Drosophila S2 tissue culture cell showing chromosome missegregation after depletion of SAC proteins. Premature exit from mitosis before proper chromosome alignment results in severe aneuploidy. Tubulin shown in green, DNA in blue and chromosomes in red.



Organoid cultures: Make your own tiny organ model

Jitka Stančíková

Laboratory of Cell and Developmental Biology, Institute of Molecular Genetics of the ASCR

Studying natural events in the most authentic and physiological conditions is an everytime wish of each researcher. Tissue cultures provide a good source of relativelly easy and fast gained results, which can be reproducible usually for many times. On the other hand tissue cultures contain only one or two cell types, lack three-dimensional organisation typical for organs and cannot be considered sufficiently complex to study relations in organism. In last years a progress in organoids culturing brought new possibilities to biomedical research. Organoids are three-dimensional structures derived from epithelium or another type of tissue. Their ability of growing in vitro is originated from stem cells isolated from original tissue which are precursors for descending cells creating the organoid. Great advantage of organoids is their organ-like behaviour and possibility to derive them from practically any organism of interest, especially human. Organoids are also appropriate for medical treatment such as developing semi-synthetic substitutions of organs (e. g. trachea) or planting organoids to damaged tissue. In conclusion organoids are superb in vitro model with a large amount of possibilities in science and medicine.

Reactivation of RNA interference in mammals – a TALE of Dicer

Eliška Svobodová

Laboratory of Epigenetic regulations, Institute of Molecular Genetics of the ASCR

Dicer is one of the key protein components of two small RNA processing pathways in mammals. Micro RNA pathway and RNA intereferece. Two Dicer isoforms are known at present. Somatic Dicer isoform has low processivity and predominantly cleaves precursors od microRNAs. Oocyte Dicer isoform found in mouse oocytes lacks N-terminal DExD helicase domain. DExD helicase aparently inhibits cleavage activity of somatic Dicer as oocyte Dicer is highly active and produces endo-siRNAs from long dsRNA substrates in mouse oocytes. This adaptation of Dicer is crucial for silencing of endogenous retroviruses in mouse oocytes. However, such Dicer isoform has also high potential for sequence specific elimination of exogenous viruses.

Our mouse model will test this hypothese and could thus lead to artificial revival of RNA intereference in mammals. RNAi as sequence specific immune response to viral infection is present in invertebrates but has been replaced by sequence independent interferon response in mammals. Our model represents evolutionary reminiscence to former immune mechanism. Mice bearing deletion of exons coding for DexD helicase in Dicer gene are already born and are being crossed and characterised. TALEN technology has an irreplaceable role in producing our mice and cell culture model systems.

New genetic model for analysis of susceptibility to *Leishmania major*

Matyáš Šíma

Laboratory of Molecular and Cellular Immunology, Institute of Molecular Genetics of the ASCR

Leishmaniasis is a complex disease caused by protozoan parasites of the genus *Leishmania*. The disease is endemic in 98 countries, more than 300 million people are at risk with 20,000-40,000 deaths annually. Mouse infection with *Leishmania major* is considered a paradigmatic model of a complex human chronic disease with immune component, however the limited number of analyzed mouse strains does not reflect heterogeneity of human population. In order to develop additional mouse model we tested response to *L. major* in the strains O20, C57BL/10Sn (B10/Sn) and C57BL/10-*H2*^{pz} (B10.O20), which carries *H2*^{pz} haplotype of the strain O20 on the B10/Sn background. Although both strains O20 and B10/Sn were resistant to *L. major,* strain B10.O20 was susceptible. Analysis of response to *L. major* in the OcB/Dem series contains 12.5% or 3.12% (strain OcB-31) of non-*H2* genes of the B10/Sn strain spread on the O20/A genome revealed that *H2*^{pz} haplotype confers susceptibility only in combination of non-*H2* genes of the B10/Sn, which were present in strains OcB-11 and OcB-31. We analyzed F_2 hybrids between OcB-31 and O20 and between OcB-43 (substrain of OcB-31) and O20. This led to mapping of three loci controlling lesion size, parasites number in liver, and hepatomegaly. B10/Sn alleles were associated with higher pathology.

Key note lecturers

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Group involvement in PhD conference