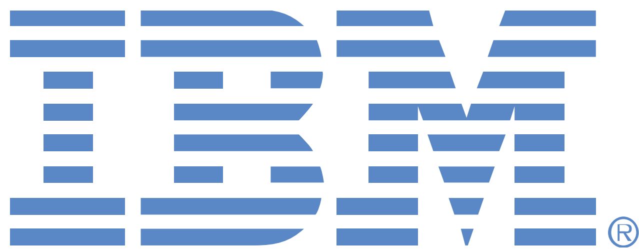
5th PhD conference

8th June 2012

Draw Us your science...!!!

Keynote lecture: Sebastian Jessberger (ETH Zürich)

Molecular control of adult neurogenesis



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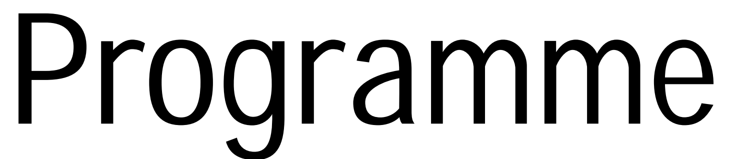

9:00 – 9:15 opening – Václav Hořejší

9:15 – 9:35 Lucie Potůčková – Nano-iPCR, a new method for sensitive detection of cytokines (Dráber Pe)

9:35 – 9:55 Pavel Marášek – Analysis of protein complexes by native PAGE (Hozák)

9:55 – 10:15 Jana Písačková – Everything you always wanted to know about protein sample for crystallization but were afraid to ask (Řezáčová)

10:15 –10:35 Lukáš Alán – Bi-PLANE FPALM nanoscopy: mitochondrial nucleoids play a central role in mitochondrial biogenesis (external PhD talk)

10:35 – 11:05 Coffee – presentations of sponsors

11:05 – 11:25 Jana Konířová – Disp3 in neural differentiation (Bartůněk)

11:25 – 11:45 Jan Mašek – The role of Tcf-3 in mouse lens development (Kozmik)

11:45 – 12:05 Markéta Černohorská – New methods for tracking of microtubules (Dráber Pa)

12:05 – 12:25 Vijay B. Arumugham – Stupidity Binding Factor in Research (Filipp)

12:25 – 13:30 Lunch

13:30 – 13:50 Polina Zjablovskaja Protein-protein interactions: Mammalian Two-Hybrid system (Bartek)

13:50 – 14:10 Jan Bražina – Viruses: Cool Tool (Anděra)

14:10 – 14:30 Anna Lounková – Factors required for replication of avian retrovirus in mammalian cells (Hejnar)

14:30 – 14:50 Denisa Kovářová – v-src-transformed chicken cell lines as a model system for identification of metastasis-associated genes (Hejnar)

14:50 – 15:20 Coffee - presentations of sponsors

15:20 – 16:20 EMBO YOUNG INVESTIGATOR LECTURE:

Sebastian Jessberger – Molecular control of adult neurogenesis (ETH Zurich)

17:00 – 00:00 Party and best presentation evaluation.

Abstracts

Nano-iPCR, a new method for sensitive detection of cytokines

Lucie Potůčková

Laboratory of Signal Transduction, Institute of Molecular Genetics of the ASCR

Cytokines are small proteins that regulate and mediate immunity, inflammation, and hematopoiesis. Several cytokines are produced by activated mast cells and are involved in a spectrum of immunologic diseases such as allergy and asthma. Reliable and simple methods are required for detection of low concentrations of cytokines in complex biological fluids. Cytokines and other immunologically active molecules are mostly detected by enzyme-linked immunosorbent assays (ELISA) and newly also by immuno-PCR (iPCR).
In this study we describe a simplified variant of iPCR where antibodies are connected with multiple DNA templates through gold nanoparticles (Au-NPs). We compared functionalized Au-NP-based iPCR (Nano-iPCR) with standard ELISA and/or iPCR for detection of interleukin (IL)-3, IL-13, stem cell factor (SCF) and tumor necrosis factor (TNF)-α.
The obtained data indicate that both Nano-iPCR and iPCR are superior in sensitivity and detection range than ELISA. Furthermore, Nano-iPCR is easier to perform than the other two methods. The combined data indicate that Nano-iPCR assays may be preferable for rapid detection of low concentrations of cytokines in complex biological fluids.

Analysis of protein complexes by native PAGE

Pavel Marášek, Dzijak R., Rohožková J. and Hozák P.

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

Most proteins exert their functions by interactions with other proteins forming protein complexes. Protein complexes organize and maintain the cellular functions on all levels – cell development and division, transport and metabolism, transcription and translation. Therfore it is a challenging task to reveal protein–protein interactions in their actual *in vivo* context.

In 1991 Hermann Schägger and Gebhard von Jagow developed a technique called „Blue native polyacrylamide gel electrophoresis“ (BN-PAGE), initially for investigation of membrane-bound protein complexes. However, over the years this method turned out to be powerful system capable of separating all kinds of protein complexes, membrane as well as soluble, even from complex mixtures. The usefulness of this technique further increased after addition of second (2D-BN/SDS-PAGE) or even third dimension (3D-BN/IEF/SDS-PAGE).

Everything you always wanted to know about protein sample for crystallization but were afraid to ask

Jana Písačková

Department of Structural Biology, Institute of Molecular Genetics

Protein crystallization is influenced by various factors. From these factors protein sample properties are the most important variable. Not only protein purity, but also homogeneity and stability determine protein crystallizability.
Various physicochemical methods are used for protein sample characterization. The combination of differential scanning fluorimetry and dynamic light scattering can be used to screen for the conditions optimal for protein sample stability, homogeneity and, therefore, crystallizability. This approach will be demonstrated on pre-crystallization analysis of single-chain antibody fragments.

External PhD talk

Bi-PLANE FPALM nanoscopy: mitochondrial nucleoids play a central role in mitochondrial biogenesis

Lukáš Alán

Laboratory of Membrane Transport Biophysics, Institute of Physiology

Mitochondria form a highly interconnected network and represent a semiautonomous organelles known for thier central role in energy metabolism and different signaling pathways, namely redox signaling. Thousands to ten thousands of the own mitochondrial (mt) DNA copies is harbored in the network (>200 000 in mammalian egg) in nucleoprotein complexes called nucleoids. Circular ds DNA (16.5 kbp in humans) encodes 13 essential genes of respiratory chain and ATP synthase. Inevitable constant mild oxidative stress as a respiration byproduct and pathogenic oxidative stress oxidize mtDNA that leads to mutations, which trigger vicious cycle of the oxidative stress due to impaired respiration, ATP synthesis, and impaired mitochondrial physiology. After exceeding a certain threshold this leads to numerous pathologies, aging and cell death.
Details of mitochondrial genetics, including mtDNA transcription and replication are still poorly understood. Investigation of these processes is very difficult, since mitochondrion has two hydrophobic lipid bilayer membranes, which must be overcome with specific probes or antibodies. Another problem is the existence of several copies of mtDNA in the nuclear chromosomes. Despite these obstacles, we have chosen microscopy as a main technique for our mitochondrial studies. Employing standard confocal microscopy we have investigated mitochondrial morphology, as a main marker of mitochondrial intactness/pathology. We have also focused on colocalizations e.g of nucleoids and mt network and in combinations of other structural features and/or mitochondrial proteins or protein complexes using superresolution microscopy (i.e. with resolution <250 nm), such as Bi-Plane FPALM (**F**luorescence **P**hotoactivation **L**ocalization **M**icroscopy) and modified dSTORM (**d**irect **S**tochastic **O**ptical **R**econstruction **M**icroscopy) technique for RNA visualization.
We focused our research on close interactions between ribosomes and nucleoids and we observed mitochondrial nucleoids as centers of ribosomal clouds. Biplane FPALM microscopy has shown that each nucleoid has its own ribosomes in very close vicinity. There were still some free ribosomes observed in the mitochondrial matrix but their amount was very low. To test whether also ribosomal RNA is close to nucleoids we applied our molecular beacon system for dSTORM *in situ* hybridization and revealed some structures corresponding to nucleoids.
Based on our findings we hypothesized that mitochondrial nucleoids are centers of translation. And taken together nucleoids are places where mitochondrial biogenesis (replication, transcription, proteosynthesis and lipid biogenesis) takes place. Another important and supporting finding is that upon fission there are no mitochondrial fragments without mtDNA and fission is probably occurring only around nucleoids.

Disp3 in neural differentiation

Jana Konířová

Laboratory of Cell Differentiation, Institute of Molecular Genetics

Neural stem (NS) cell lines could be used for the investigation of basic molecular, cellular, and developmental processes in the central nervous system. It was shown that NS cells can be derived from embryonic stem (ES) cells and from cells found in the forebrain and subventricular zone areas of the embryonic or adult brain, respectively. These cells can be stably propagated in vitro in clusters called neurospheres. In the presence of fibroblast growth factor 2 (FGF2) and epidermal growth factor (EGF) it is possible to establish adherent symmetrical expandable NS cell lines, which retain the ability to differentiate into neurons, astrocytes and oligodendrocytes.
Recently, we have identified Dispatched 3 (DISP3) as a gene directly regulated by thyroid hormone. Thyroid hormone is known to regulate CNS development by influencing neural stem cells proliferation and differentiation. We have also found relatively high level of DISP3 in two NS cell lines (NS-5 and O4-ANS) as well as in other neural tissues. We are able to validate the regulation of DISP3 in NS cells by thyroid hormone. Moreover, we have found that the levels of DISP3 dramatically change upon differentiation of NS cells into neurons, astrocytes and oligodendrocytes. Based on these results we have created stably transfected NS cell lines with up or down regulated levels of DISP3 to better understand the role of DISP3 in self-renewal and/or differentiation of neural stem cells.

The role of Tcf-3 in mouse lens development

Jan Mašek

Laboratory of Trascriptional Regulation, Institute of Molecular Genetics

A tight control of Wnt/β-catenin signaling is essential for the formationof the vertebrate eye. In mouse, down-regulation of canonical Wnt signaling in the lens surface ectoderm is a prerequisite for proper cell specification in the lens placode. Tcf/Lef transcription factors are responsible for the transcriptional response to Wnt signaling.
We study phenotypic changes in the embryonic eye upon conditional inactivation of Tcf7L1 (Tcf-3) using Lens-specific Cre driver. Deletion of Tcf7l1 in the lens epithelium at E9.5-10 by Le-Cre led to defects in proliferation resulting in dramatic reduction of the lens size without an increased apoptosis. Apart from the smaller size, the organization of the lens and the expression of specific genetic markers of differentiation such as γ-crystalline, FoxE3, Prox1 and cMaf appeared normal. The lens tissue almost disappeared in adult Tcf7L1-/- mutants.

New methods for tracking of microtubules

Markéta Černohorská, Vinopal S., Dráber Pa.

Laboratory of Biology of Cytoskeleton, Institute of Molecular Genetics

Microtubules are highly dynamic polymers that rapidly switch between states of growth, shortening, and pause, a behaviour known as dynamic instability. These dynamic events take place predominantly on microtubule plus ends and are critical for many microtubule functions such as intracellular trafficking, signaling and adhesions, polarization and migration, and segregation of chromosomes during cell division. Quantification of microtubule dynamics can be used as a read-out for functional characterization of new microtubule modulators.
The traditional way of annotating microtubules manually is becoming a daunting task. Thus, the goal is to research and develop an efficient, reliable, and rapid microtubules tracking technique. We have established two methods for automated tracking of microtubules in our laboratory. Features of both the home-made and the recently published algorithms will be demonstrated on one of our currently running projects investigating the role of GIT-PIX/PAK signaling complex in the regulation of microtubules.

"Stupidity Binding Factor in Research"

Vijay B. Arumugham

Laboratory of Immunobiology, Institute of Molecular Genetics

Are we all stupid? Can we use the power of stupidity to do research?Will it help? Think different.

Studying protein-protein interactions: mammalian two-hybrid system

Polina Zjablovskaja

Laboratory of Genome Integrity, Institute of Molecular Genetics

The study of proteins and their functions plays a central role in understanding both, cells and organisms. One way to study functions of protein is to identify its interacting partners because the majority of the proteins work as a part of complexes. A lot of approaches have been developed for identification of protein-protein interactions. One of the most utilized is yeast two-hybrid system (Y2H). *In short: two proteins are fused with different parts of split transcription factor and expressed in the cells. Interaction between this two proteins leads to restoration of transcription factor functions and expression of reporter gene.*
As a complementation to Y2H, mammalian two-hybrid system was developed which was based on the same strategy as Y2H. Clear advantage of this method is possibility to study protein interactions in nearly native conditions. Moreover, several new modifications of this method allow monitoring the temporal, spatial and functional modulation of protein–protein associations. All this makes mammalian two-hybrid a powerful tool to investigate interprotein interactions.

Viruses: a cool tool

Jan Bražina

Laboratory of Cell Signaling and Apoptosis, Institute of Molecular Genetics AS CR

Transient or stable gene knockdown or forced expression of exogenous cDNA have become one of the fundamental methodical approaches in the biological sciences. Besides the classical transient siRNA or plasmid transfections, several viral systems have been established, such as recombinant retroviral, lentiviral or adenoviral vectors. Each of them is applicable under different circumstances. Unlike classical transfection the viral transduction has got several advantages: higher efficiency, broader range of various cell types (viral amphotropism), stability via genome integration, possible inducibility etc. However, there are also some disadvantages and limitations of viral transduction in contrast to transfection (e.g. safety, limited size of inserts, etc.) or when particular viral systems are compared among themselves (capacity of the viral capsid, amphotropism). Thus, a choice of the right gene knockdown method should be well-judged. As our laboratory uses several of these viral transduction systems in almost all running projects, I shall present both their overview and practical examples.

Factors required for replication of avian retrovirus in mammalian cells

Anna Lounková, Trejbalová K., Geryk J., Šenigl F., Dráberová E., Hejnar J., Svoboda J.

Laboratory of Viral and Cellular Genetics, Institute of Molecular Genetics

Rous sarcoma virus (RSV), an avian retrovirus, can transform mammalian cells. Although RSV-infected mammalian cells integrate the provirus into their genome (and become virogenic cells), there is no virus production. However, this block can be overcome by fusion of virogenic mammalian cells with permissive chicken fibroblasts. Our study is focused on molecular characterization of the described virus rescue.

In our experiments, the transforming virus rescued from the virogenic hamster cells was detectable on the third or fourth day after the fusion. The specific viral envelope glycoprotein was identified by immunofluorescent labeling in 20 % of polykaryons on the second day after the fusion. In contrast, the glycoprotein was not detected in mononuclear cells.

To determine the level of viral RNA and mRNAs in virogenic and fused cell lines, we used SYBR green based real time RT-PCR. The amount of viral unspliced RNA, which serve as the template for translation of polyproteins Gag and Gag-Pol and also as genomic RNA, was clearly lower in virogenic hamster cells compared to infected avian cells, but did not change after the fusion. The amount of *src* mRNA in virogenic cells reached almost the same level as in the infected avian cells and formed the majority of total viral RNA. In contrast, *env* mRNA synthesis was kept very low in virogenic cells, but increased ten times after the fusion. We also identified anomalously double spliced RNAs, which were generated by processing *env* mRNA using cryptic 5′ splice sites. Measurement of cytoplasmic and nuclear fractions revealed less efficient RNA export of unspliced and *env* mRNA in virogenic cells than in the fused cells.

v-*src*-transformed chicken cell lines as a model system for identification of metastasis-associated genes

Denisa Kovářová, Jan Kosla, Kateřina Trejbalová, Jiří Plachý, Jiří Hejnar

Laboratory of Viral and Cellular Genetics, Institute of Molecular Genetics

Metastatic spread of cells from a primary tumour to distant tissues and organs represents a critical step in the tumour progression. Not all genes involved in this process have been identified. To find new players in cancer progression we use our original model system of chicken v-*src*-transformed tumour cell line PR9692. Rare subclones of this cell line have lost their ability to induce metastases after inoculation into syngeneic chickens. We compared the expression profile of metastatic and non-metastatic cells using oligonucleotide microarrays, assuming that metastasis-associated genes are among those with differential expresssion.
In this study integrin alpha 4 (ITGA4) and homeodomain only protein X (HOPX) were assessed for their involvement in metastasis formation using knock-down induced by plasmid-delivered shRNAs. We chose several clones that demonstrated the most important downregulation and these clones were further analysed. We show that clones of both ITGA4 and HOPX knock-downs display reduced migration *in vitro*. Moreover downregulation of ITGA4 gene led to a decrement in the incidence of metastases *in vivo*. In case of HOPX knock-down we observed even a reduction of the primary tumour formation. This indicates that ITGA4 and HOPX play an essential role in maintaining the malignant phenotype. Their role in the tumour progression will be further characterized.

EMBO Young Investigator Lecture

Molecular control of adult neurogenesis

Sebastian Jessberger

Institute of Molecular Health Sciences, Dept. of Biology, Swiss Federal Institute of Technology (ETH) Zurich, Switzerland

Adult neurogenesis is critically involved in certain forms of learning and memory and has been associated with neuro-psychiatric diseases such as major depression and epilepsy. We used unbiased gene expression profiling of adult NSCs and their neuronal progeny directly isolated from the adult brain i) to identify molecular regulators of stem cell activity and ii) to analyze how gene expression signatures differ among NSCs and immature neurons between the two neurogenic regions of the adult brain, the hippocampal dentate gyrus (DG) and the subventricular zone (SVZ). With this approach we found that the transcriptomes of NSCs from the adult DG and SVZ are highly similar but that gene expression profiles substantially diverge in immature neurons. Furthermore, I will provide evidence for the functional significance of several pathways newly identified by our transcriptome analyses and present our current efforts aiming to translate these findings into the development of novel treatment strategies for therapeutic manipulation of neurogenesis in neuro-psychiatric diseases.

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IMG

Group involvement in PhD conference

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