6th PhD Conference

IMG

Keynote lecture:

Monica Bettencourt-Dias,
Instituto Gulbenkian de Ciência,
Oeiras, Portugal

Invited PhD talk:

Lukáš Pastorek,
University of Economics,
Prague, Czech Republic

Institute of Molecular Genetics of the ASCR, v. v. i.

7th JUNE 2013

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9:00 - 9:15	Opening - Václav Hořejší
9:15 - 9:35	Jiří Pergner: The Good, the Bad and the OPSIN. (Kozmik)
9:35 - 9:55	Zuzana Hájková: New Method for Exploring Chemotaxis of Mast Cells. (Dráber Pa)
9:55 - 10:15	Ondřej Ballek: T Cell Stronghold: How to Smuggle Trojan Horse in? (Filipp)
10:15 - 10:35	Blanka Gabajová: Aptamers and *in vitro* Evolution. (Bartek)
10:35 - 10:55	Coffee break + presentations of sponsors
10:55 - 12:00	**EMBO YOUNG INVESTIGATOR LECTURE:** **Monica Bettencourt-Dias: Centrosome Number Control: Right Time, Right Place and Only Once (Instituto Gulbenkian de Ciência, Oeiras, Portugal)**
12:00 - 13:00	Lunch
13:00 - 13:20	Yahya Sohrabi: Enigmatic *H2*^{pz} Haplotype in Susceptibility to *Leishmania major.* (Lipoldová)
13:20 - 13:40	Jitka Stančíková: NKD1- CreER^{T2} mouse: A New Tool for Recombination in Wnt Responsive Cells of Mouse Intestine and Liver. (Kořínek)
13:40 - 14:00	Petr Těšina: Structural Study of LEDGF/p75 Binding Partners. (Řezáčová)
14:00 - 14:20	Coffee break
14:20 - 14:40	Alena Hájková: Relationship of Metabotropic Glutamate Receptor 1 Splice Variants. (Blahoš)
14:40 - 15:00	Ivan Štěpánek: Regulatory T Cells: Wanted Dead Not Alive. (Reiniš)
15:00 - 15:20	Lenka Kyjacová: Magnetic-activated Cell Sorting: a Useful Method for Separation of Low Abundant Cell Subpopulations. (Bartek)
15:20 - 15:30	Voting for the best presentation
15:30 - 16:20	EXTERNAL PhD TALK: **Lukáš Pastorek: Mean – Standard – Normality and P-Value**
17:00 - 00:00	Party and best presentation evaluation.

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Abstracts

The good, the bad and THE OPSIN.

Jiří Pergner

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

Opsins are proteins belonging to G protein coupled receptor superfamily (GPCR). The opsins consist from protein moiety and cofactor retinaldehyde (mostly 11-cis-retinal) and have the ability to detect light. The opsins are exclusivelly used in visual and non visual organs of all metazoans. GPCR use after activation signal transduction cascade involving trimeric G proteins and different second messengers (e.g. cAMP, cGMP, Ca2+), thus leading to variable cellular responses. There has been identified more than 800 opsin gene sequences in different animals so far, but less then 30 of them have been connected to proper signal transduction cascade.

In our lab we have established semi-high throughput method for opsin-G protein-second messenger cascade identification based on commercial available cell line. Identification of opsin-G protein-second messenger signal transduction cascade is important not only for understanding opsin and vision evolution, but also for studying GPCR signalling mechanism in general.

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New method for exploring chemotaxis of mast cells.

Zuzana Hájková

Laboratory of Biology of Cytoskeleton, Institute of Molecular Genetics of the ASCR

Mast cells are immune system cells that play an essential role in innate immunity, allergy, and inflammation. Their committed progenitors, derived from hematopoietic progenitors, are released from the bone marrow to circulate in the bloodstream. Subsequently, they migrate into peripheral tissues to undergo terminal maturation under the control of locally produced cytokines and growth factors. Chemotaxis, the chemoattractant-directed migration, of mature mast cells or their progenitors might be one of the key mechanisms responsible for their local accumulation. Insight of the molecular mechanisms that lead to the mast cell migration is essential for better understanding of mast cell function in health and disease. Various methods for studying cell migration have been established, but they have some limitations for exploring chemotaxis of mast cells. Here we report on a new technique that makes it possible to study directed migration of non-adherent mast cells to chemoattractant. The experimental setup utilises real-time imaging that enables evaluation of various parameters of cell movement. Presented method is a valuable tool for exploring chemotaxis of mast cells, and could be also used for studying the migration of other cell types.

T-cell stronghold: how to smuggle a Trojan horse in?

Ondřej Ballek

Laboratory of Immunobiology, Institute of Molecular Genetics of the ASCR

T lymphocytes are immune cells of hematopoietic origin responsible for the adaptive arm of immunity. Their main job is the recognition of pathogen-specific antigens and subsequent activation of other immune cells, removal of virus-infected cells or suppression of other effector T cells. The insight into molecular mechanisms controlling these functions is gained mainly by manipulating lymphocytes using a variety of protocols for gene delivery. Unfortunately, in case of using freshly isolated primary cells as targets, this task is often very complicated. For example, our cells of interest are murine primary $CD4^+$ T cells that, especially in their naïve state, are very resistant to transgenesis. Metaphorically, they act like a stronghold into which it is very difficult to break in. For this purpose, several protocols were specifically established and are used with various degree of success. The main problem is the cell viability, transduction efficiency and how these protocols affect the function of target cells. To bypass these limitations, a transgenic mouse which expresses Coxsackie Adenovirus Receptor (CAR) on the surface of T cells was developed several years ago. The adenoviral infection can thus serve as an efficient tool (a Trojan horse) to smuggle into T cells several types of DNA recombinant constructs aiming at the overexpression or downregulation of our favourite gene. The advantage of this useful methods and its direct comparison with alternative approaches for gene delivery into naïve lymphocytes will be presented.

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Aptamers and *in vitro* evolution.

Blanka Gabajová

Laboratory of Genome Integrity, Institute of Molecular Genetics of the ASCR

Aptamers, short single-stranded DNA or RNA molecules, folded into stabile structures, are able to recognize a wide range of targets with high afinity and specificity. They are usually generated by *in vitro* partitioning technology called SELEX (systematic evolution of ligands by exponential enrichment). Compared to antibodies, aptamers possess plenty of advantages such as high stability and low immunogenicity. Thus, aptamers have diagnostic and therapeutic potential. They can be prepared against simple targets, e.g. proteins or against complex targets such as live cancer cells. The latter alternative is able to provide several aptamers recognizing molecules on the surface of target cells. These aptamers can be further used for identification of the molecules they bind to, thus providing insight into the mechanism of cancer development. By this method, we generated and identified aptamers against PC3 prostate cancer cell line.

EMBO Young Investigator Lecture

Centrosome Number Control: right time, right place and only once.

Monica Bettencourt-Dias

Instituto Gulbenkian de Ciência, Oeiras, Portugal

Centrosomes are the major microtubule organizer in animal cells, while cilia and flagella are important in signalling and motility. Centrosomes undergo duplication once every cell cycle, so that their number remains stable, rather like the genetic material. Abnormalities in centrosome and cilia number and structure occur in many cancers and are associated with genomic instability. An understanding of the pathways involved in the regulation of microtubule-organising centers formation will be invaluable to generate diagnostic and prognostic markers, and provide novel therapeutic targets. I will discuss research in my laboratory concerning the regulation of major molecular players in centrosome biogenesis.

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Enigmatic *H2^{pz} haplotype* in susceptibility to *Leishmania major*.

Yahya Sohrabi

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

Leishmaniasis is widely used as an important model of interaction between immune system and the infectious agent. Mouse model of leishmaniasis also enables studying of the genetic control of susceptibility to the disease. Susceptibility to *L. major* is not due to a single immunological mechanism, but rather can result from effects of multiple genes that interact in a functional network. Presentation of the antigen by major histocompatibility complex (MHC) molecules to T-cell receptors (TCR) controls the onset of immune response. To study the role of *H2* molecules in genetic controls of immune response, B10.O20 strain was prepared. B10.O20 is a *H2* congenic strain on the C57BL/10 (B10/Sn) background and carries the *H2^{pz}* haplotype derived from strain O20/A (O20). Both parental strains B10/Sn and O20 mice are resistant to leishmaniasis whereas surprisingly B10.O20 is susceptible. B10.O20 mice develop higher skin and visceral pathology. We hypothesize that interaction between *H2* part of O20 strain and non *H2* part of B10/Sn strain may cause susceptibility of B10.O20 mice to lesishmaniasis. To study the effects of non *H2* part of mouse genome in controlling immune response, OcB/Dem series were prepared from B10.O20 mice as a donor strain on O20 background. When OcB/Dem series were infected with *L. major*, the only strain that developed pathology was OcB-31. OcB-31 strain, B10.O20 and O20 strains all carry *H2^{pz}*, therefore, non *H2* part of the genome is responsible for susceptibly to leishmaniasis in OcB-31 mice. Development of the mouse model to study role of *H2* and non *H2* part of the genome in susceptibly to disease (leishmaniasis as an example) will be discussed in this presentation.

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NKD1- CreER^{T2} mouse: A New Tool for Recombination in Wnt Responsive Cells of Mouse Intestine and Liver.

Jitka Stančíková

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

Wnt signaling pathway plays a crucial role in ontogenesis and development of all metazoans. In adult mammals, the Wnt signaling pathway is required for the maintenance of the intestinal homeostasis and establishment of proper hepatic zonation. In contrary to that, aberrant activation of the Wnt pathway leads to neoplasia and cancer development, notably in the intestine and liver.
To investigate the role of the Wnt pathway in gut epithelium homeostasis and its malignant transformation we employed chromatin immunoprecipitation method (ChIP) in combination with DNA microarrays (so-called ChIP-on-chip) to identify genes regulated by the Wnt signaling. One of the most prominent targets was the NKD1 (Naked Cuticle Homolog 1) gene; previously identified as a Wnt-induced intracellular negative regulator of the canonical Wnt signaling.
With use of BAC recombineering, we generated mice with CreER^{T2} recombinase produced corresponding to the gene NKD1 (NKD1-CreER^{T2}) expression. Comparing the natural NKD1 expression with transgenic Cre in NKD1-Cre x Rosa-lacZ reporter strain hybrids proved that the transgenic mouse produces Cre in NKD1^{+} cells only. Two of the most interesting sites of the NKD1-CreER^{T2} expression in adult mice are perivenous hepatocytes and intestinal crypt compartment, which was confirmed by the expression profiling. New mouse strain NKD1-Cre ER^{T2} is therefore a unique tool for gene manipulation particularly in hepatocytes localized in the perivenous zone.

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Structural Study of LEDGF/p75 Binding Partners.

Petr Těšina

Laboratory of Structural Biology**,** Institute of Molecular Genetics of the ASCR

Lens epithelium-derived growth factor p75 (LEDGF/p75) is a prominent cellular cofactor for human immunodeficiency virus (HIV) integration. LEDGF/p75 tethers the preintegration complex to the host chromosome and this process is crucial for HIV replication. HIV integrase interacts with the C-terminal part of LEDGF/p75, region designated integrase-binding domain (IBD, amino acids residues 347 - 429). Interaction interface between HIV integrase and LEDGF/p75 became an attractive target for design of small molecule inhibitors blocking this interaction.
As a transcriptional co-activator, LEDGF/p75 is implicated not only in HIV replication, but also in human cancer and autoimmunity. The LEDGF/p75 was shown to interact through its IBD with several cellular proteins and recent evidence implies that LEDGF/p75 is a general adaptor protein tethering various factors to chromatin.
In this work, we set to prepare two LEDGF/p75 physiological binding partners JPO2 and pogo transposable element (pogZ). The aim of our study is to obtain structural information on the LEDGF/p75 interaction with its physiological binding partners JPO2 and pogZ, respectively. Such structural information is essential for understanding the LEDGF/p75 biological role and might help in design of inhibitors selectively blocking interaction with HIV integrase while not interfering with the LEDGF/p75 biological function.

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Relationship of Metabotropic Glutamate Receptor 1 Splice Variants.

Alena Hájková

Laboratory of Molecular Pharmacology, Institute of Molecular Genetics of the ASCR

The Metabotropic Glutamate Receptors 1 (mGluR1) modulate excitatory neurotransmission. Functional receptors are covalently linked dimers localized post-synaptically on the cell surface. Alternative splicing of mGluR1 gene results in expression of a long variant mGluR1a and short mGluR1b that vary within their intracellular C-termini. Their localization and signalization properties differ when expressed alone. Carboxy terminus of both splice variants contain endoplasmatic reticulum (ER) retention signal RRKK which has to be neutralized for the receptor to reach cell surface. In mGluR1a this RRKK sequence is masked by its own long carboxy terminus. On the other hand, mGluR1b is reatined in ER. In our previous studies we showed that mGluR1a and mGluR1b form heterodimers in transfected HEK293 cells. Now we extended our observation of mGluR1 splice variant heterodimerization in vivo. Moreover, we show that trafficking of mGluR1b in neurons is dependent on association with mGluR1a. Thus, heterodimers mGluR1a-mGluR1b have unique properties distinct from the homodimeric receptor complexes.

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Regulatory T Cells: Wanted Dead Not Alive.

Ivan Štěpánek

Laboratory of Molecular Pharmacology**,** Institute of Molecular Genetics of the ASCR

$CD4^+CD25^+Foxp3^+$ regulatory T cells (Treg) play a critical role in ensuring tolerance, but their presence can be counterproductive to the attempts of inducing antitumour immunity. Treg depletion with specific antibodies targeting the CD25 molecule is under investigation as a tool to increase the efficacy of anti-tumour immunotherapy. We focused on depletion of Treg by anti-CD25 mAb (PC61) and its possible additive effect on immunotherapy of the TC-1 murine tumours targeting invariant NKT (iNKT) cells with their activation ligand α-GalCer. Unexpectedly, depletion of Treg by PC61 mAb had no additive effect to the iNKT cell activation on the tumour growth. We tried to determine in more detail if anti-CD25 antibody (PC61) interferes with the iNKT cell stimulation. In these experiments we employed DEREG mice expressing diphtheria toxin receptor under the FoxP3 promoter. This system allows specific Treg depletion. By comparison of results obtained in DEREG mice to the results from the PC61 antibody-treated wild type mice, we could distinguish the specific effects of Treg depletion from further effects of PC61 mAb. Our experiments showed that PC61 mAb not only depleted Treg, but also inhibited activated iNKT cells, which also express CD25. PC61 mAb limited proliferation of iNKT cells after activation, decreased production of IFNγ by activated iNKT cells and was associated with downregulation of the early surface activation marker CD69. These data demonstrate that anti-CD25 mAb treatment targeting Treg can be controversial and its efficacy is dependent on the particular treatment setting. Moreover, alternative methods for Treg depletion, such as metronomic cyclophosphamide treatment, should be considered.

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Magnetic-activated Cell Sorting: a Useful Method for Separation of Low Abundant Cell Subpopulations.

Lenka Kyjacová

Laboratory of Genome Integrity, Institute of Molecular Genetics of the ASCR

Fluorescence activated cell sorting (FACS) and magnetic-activated cell sorting (MACS) are both well-established methods for isolation of subpopulations from cell mixtures. Inspite of numerous benefits, FACS is often limited by its low separation capacity, demanding sample preparation and time-consuming isolation of rare cells. On the other hand, MACS represents a powerful technique for cell isolation excellently dealing with the problems mentioned above. Firstly, cells are incubated with magnetic nanoparticles coated with antibodies against specific surface antigen. Particular cell type is subsequently depleted or retained from the mixture using a strong magnetic field. Thus, magnetic cell sorting system allows cells to be separated more effectively, easily, and faster in comparison to commonly used sorting methods. Magnetic cell sorting has become a powerful technique for cell isolation not only in immunology, stem cell biology and neuroscience, but also in cancer research.

Prostate cancer is one of the most common cancer in Europe. In up to one third of all cases, prostate cancer recurs as a secondary tumor certain time after the fractionated irradiation (fIR) pointing to the existence of radioresistant cells escaping the radiotherapy. In our study, we treated prostate metastatic cell lines DU145, PC-3, LNCaP and 22RV1 with clinically relevant daily fractions of ionizing radiation (2 Gy) for 10 and 35 days. fIR resulted in the massive loss of adhesion in all four cell lines as a consequence of sequential events leading to apoptosis. Surprisingly, in the case of the most tumorigenic lines (DU145 and PC-3), we were able to detect a small fraction of anoikis-resistant non-adherent viable cells. Using FACS method for their isolation, we were facing several problems concerning long duration and low effectivity of the sorting process and high heterogenity of the sorted population. When magnetic cell sorting was employed, we were able to eliminate almost all mentioned problems and gained more homogenous population for further analysis in shorter time.

Thus, MACS could represent, in particular situations, a more suitable alternative for cell sorting in comparison to the standard cell separation methods.

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Mean – Standard – Normality and P-Value.

Lukáš Pastorek

University of Economics, Prague, Czech Republic

What is standard deviation anyway? What is its connection to the arithmetic mean? What does it mean to get p-value less than 0.05? Arithmetic mean, standard deviation and p-value are basic statistical concepts, which are widely used, but many times with very small user`s comprehension and vague idea of their real meaning. P-value is one of themost important scientific concepts which spread across the various fields of science during the last century. But why we use it? + BONUS simulation: Have you ever seen artifical neural network?

This work was supported by project IGA VSE F4/17/2013 IG410023.

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Group involvement in PhD conference