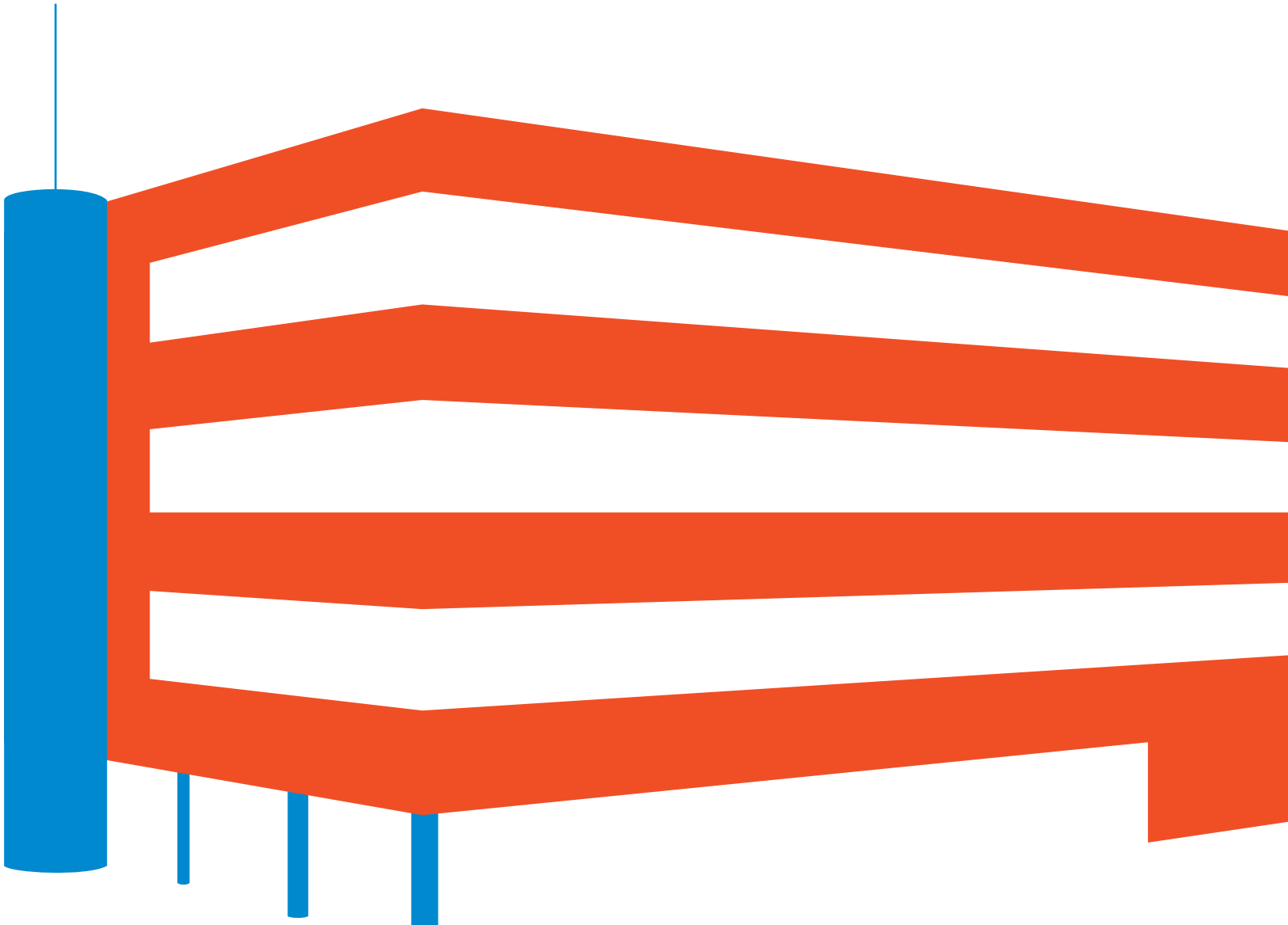
IMG

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

2009-2010



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Introduction

The period 2009-10 was rather eventful for IMG, as well as for the entire Academy of Sciences (AS) - partly due to the economic crisis generally, but mainly because of incompetent decisions made by the Council for Research, Development and Innovations of the Czech Government, which threatened to bring extremely serious consequences for the AS future. Fortunately, the AS employees demonstrated very strong opposition and in their protests received substantial support also from the public and from journalists. Many of us, unfortunately, lost hundreds of hours of valuable time in these "battles". Despite this, I believe that all this effort was not in vain and that all will come right in the end. I was especially pleased by the active interest of young researchers (namely students) of the platform "Science Alive".

In the past two years we were successful in what is our *raison d'être* – good science. We again published a number of scientific reports in peer-reviewed international scientific journals; we transferred a number of practically applicable products to our partner spin-off companies.

Of high importance for scientific life at our Institute was the organization of many scientific seminars and lectures given by our researchers and our guests. Our new conference hall (since January 2009 bearing the name "Milan Hašek Auditorium" in honour of Milan Hašek, the founder of our Institute) hosted several international conferences, some also organized by other AS institutes from the campus. I would like to mention the especially successful "EMBO Workshop on Mitochondria, Apoptosis and Cancer" held in October 2009, "Centennial Retrovirus Meeting" (May 2010), "The Second European Chemical Biology Symposium" (May 2010), "EMBO Young Scientists Forum" (June 2010) and "52nd Symposium of the Society for Histochemistry" (September 2010).

We completed the reconstruction of the animal facility for non-mouse models (mainly chicken), as well as the construction of a kindergarten and gym adjacent to our main building that will serve also our colleagues from other institutes of the campus.

We started the first phase of reconstruction of our chicken breeding facility in Koleč.

It is gratifying that our researchers repeatedly obtain rich and prestigious local and international grants to support their experiments aimed at discovering the still-so-many secrets of cells and tissues that decide about our health or disease.

At present, 23 research groups of the Institute are dealing with the topics covering molecular and cellular immunology, molecular and cellular oncology, cell biology of the nucleus, cytoskeleton, functional genomics and bioinformatics, study of oncogenes, molecular biology of development, structural biology and mechanisms of receptor signalling. An essential part of our Institute is represented by 95 doctoral students and 31 undergraduate students. A number of our scientists also act as university teachers (e.g. eight as professors and nine as associate professors).

Although we consider basic research as the highest priority, we are happy that some "by-products" of our basic research have practical importance. We are collaborating with several prospering biotech spin-off companies that have been born at the Institute in recent years.

In 2009-10, the Institute scientists were again authors or co-authors of publications in a number of prestigious international journals (e.g. Nature, Science, Molecular and Cellular Proteomics, Nucleic Acids Research, Human Molecular Genetics, Cancer Research, Blood, PLoS Pathogens, Cancer Cell, Current Biology, EMBO Reports, Journal of Cell Biology, Journal of Immunology, Oncogene, Cellular and Molecular Life Sciences, Journal of Cell Science, EMBO Journal, Journal of Virology, Nature Protocols).

The high standing of the Institute researchers is testified by awards and prizes; in 2009 Jiří Forejt was honoured with the Prize of the Minister of Education, Youth and Sports for outstanding results in research, experimental development and innovations for 2009; the team of Jiří Forejt laboratory (Zdeněk Trachtulec, Soňa Gregorová, Petr Jansa, David Homolka, Ondřej Mihola) was awarded the Prize of the Academy of Sciences of the Czech Republic; Ondřej Ballek obtained the Prize for the best diploma thesis awarded by the Dean of Charles University, Prague; Daniel Smrž (and the laboratory of Petr Dráber) received the Prize of the Czech Immunological Society for the best paper by a young immunologist in 2008; Petr Heneberg obtained the Bolzano Award awarded by the Rector of Charles University; Václav Pačes received the Prize of the Economia publishing house; Václav Hořejší was honoured with The Sir Hans Krebs Lecture and Medal awarded by FEBS; Daniel Smrž obtained the Award of the Czech Society for Analytical Cytology; Jiří Bartek was awarded The Shay Shacknai Prize for Cancer Research by the Hebrew University of Jerusalem, and the Medal of the Faculty of Medicine, Charles University. In 2010 Jan Svoboda was honoured with the National Prize of the Czech Government "Česká hlava" (Czech Brains); Martina Huranová obtained from the Hlávka Foundation the Josef Hlávka Award for the best University students and graduates; Ondřej Mihola received the Arnold Beckman Prize for the best publication in the field of Genetics from Beckman Coulter CR and the Czech Society for Biochemistry and Molecular Biology; Václav Pačes was awarded the Medal of Emil Votoček by the Institute of Chemical Technology, Prague; Ondřej Mihola obtained the Scopus Award for year 2009 awarded by the prominent scientific publisher Elsevier BV; Jiří Bartek received the Neuron 2010 for life-long merits in medicine awarded by Karel Janeček Foundation.

Despite the potential funding problems, the development of our Institute complex is continuing successfully and I believe we can look forward to an exciting future.

Václav Hořejší
Director

Václav Hořejší
Director

Petr Dráber
Deputy Director

Jiří Špička
Deputy Director for Economy

Jan Rajnoch
Deputy Director for
BIOCEV Project Implementation

Institute Representatives

Miroslav Flieger
Member of the Academy Council of the ASCR
Chairman of the Supervisory Board

Jiří Forejt
Chairman of the Institute Council

Šárka Takáčová
Institute Secretary

A Brief History of the IMG

Milan Hašek (* 1925, † 1984)
1st Director

The Institute of Molecular Genetics, Academy of Sciences of the Czech Republic (IMG), is located on the southern outskirts of Prague, capital of the Czech Republic.

The history of the Institute started in 1953 with the establishment of the Department of Experimental Biology and Genetics of the Institute of Biology of the Czechoslovak Academy of Sciences, headed since then by Milan Hašek, co-discoverer of immunological tolerance. In 1961, the Department was transformed into the Institute of Experimental Biology and Genetics (IEBG), with Milan Hašek as its Director until 1970. The sixties of the last century were the "golden age" of the Institute, represented besides Hašek e.g. by Pavol and Juraj Iványi, Jan Klein, Jan Svoboda, etc. The end of the "Prague Spring" after August 1968 closed this famous era – many promising young scientists had emigrated (and were very successful at their new institutions abroad). In 1977, IEBG was re-organized and renamed Institute of Molecular Genetics of the Czechoslovak Academy of Sciences (IMG); Josef Říman was appointed its Director. Among the achievements of the otherwise difficult seventies and eighties were co-discovery of reverse transcriptase (J. Říman), discovery of virogeny (J. Svoboda) or sequencing of one of the first viral genomes (V. Pačes). After 1989, the Institute was headed by Jan Svoboda (1991-1999), Václav Pačes (1999-2005) and Václav Hořejší (2005-present). In the period 1964-2006, the Institute was divided between two distant locations. After completion of a modern new building for IMG in 2007, both parts moved together and the new premises are now hosting more than 350 employees and students.

IMG and Its Surroundings

The Prague-Krč Campus of Biomedical Institutes of the Academy of Sciences
IMG is located on the Krč campus also hosting the Institute of Microbiology, Institute of Physiology, Institute of Experimental Medicine, Institute of Biotechnology and part of the Institute of Animal Physiology and Genetics, in total over 1200 researchers and students. In close proximity to the site is also located the Institute of Clinical and Experimental Medicine (IKEM) and Thomayer Hospital. The campus lies near a major natural park (Krč forest), and is easily reachable by car or public transportation.

Prague – A City of History, Culture and Science
Situated on the Vltava (Moldau) river Prague has been the political, cultural, and economic centre of the Czech state for over 1000 years. The city is home to nearly 1.3 million people. Prague is widely considered to be one of the most beautiful cities in Europe and belongs to the most visited cities on the continent. Since 1992, the historic centre of Prague has been included in the UNESCO list of World Heritage Sites. Prague also has a long-standing tradition in science. Founded in 1348, Charles University is the oldest University in Central Europe. At present, Prague is the seat of eight public Universities, the student population being more than 100,000. There are also 36 institutes of the Academy of Sciences and a number of other research institutions.






Laboratory of Cell Signalling and Apoptosis

Death receptors, TRAIL, Daxx, cancer, cell death

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Our group is interested in deciphering the signalling pathways leading to programmed death of cancer cells including cancer stem cells and uncovering mechanisms participating in regulation of these pathways. In our major focus stands TRAIL (TNFα-Related Apoptosis Inducing Ligand), a ligand from the TNFα family capable of inducing apoptosis of a number of transformed cells and not being harmful to the normal ones. TRAIL-induced apoptosis of sensitive cells is triggered by its interaction with TRAIL-R1/DR4 and/or TRAIL-R2/DR5. These receptors contain an α-helical protein-protein interaction domain called the death domain and belong together with Fas/CD95 or TNFR1 to the subfamily of TNFR receptors named "death receptors". We analyse several aspects of TRAIL's biological activities such as the role of endocytosis in TRAIL ligand-receptor(s) Death-Inducing Signalling Complex (DISC) formation and activation, TRAIL signalling in human embryonic stem cells (hESCs) and from them derived somatic progenitors or the effect of overexpressed/activated oncogenes such as c-Myc on TRAIL-induced apoptosis. In our collaborative projects we uncovered two novel drugs sensitizing resistant cancer cells to TRAIL-induced apoptosis and participated in the analysis of multiple aspects of TRAIL-induced signalling in leukaemia and colon carcinoma cells. In our other death receptors-related project we characterize molecular and expression patterns of the Death Receptor 6 (DR6), which can participate in the regulation of T- and B-cell activation. We have discovered that posttranslational modifications of DR6 can affect the cellular localization of this highly glycosylated and palmitoylated receptor, and we currently analyse regulation of DR6 expression in T cells. Part of our group also analyses molecular and functional properties of the for embryonic development essential adapter protein Daxx. Daxx apparently participates in stress- and Fas/CD95-triggered apoptosis, and it is also involved in the regulation of multiple other processes such as transcription or cell cycle. We currently characterize functional consequences of its interaction with several new Daxx-binding proteins such as Brg1 or BAP1 and in collaboration we assess the role of Daxx in DNA damage response.

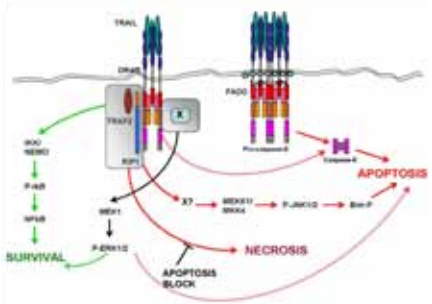

Fig. 1. Signalling pathways triggered by activated TRAIL receptors.

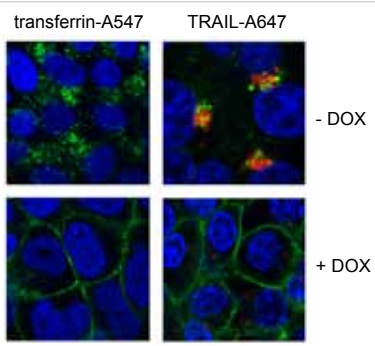

Fig. 2. Endocytosis of the activated TRAIL receptor is blocked by inducible expression of the dominant-negative dynamin-1. In green either Alexa547-labelled transferrin or TRAIL, in red lysosomal marker LAMP-1, +DOX stands for doxycycline-mediated induction of the dominant-negative dynamin-1 expression.

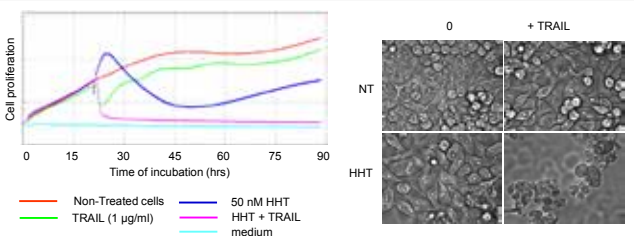

Fig. 3. Natural alkaloid homoharringtonine (HHT) enhances TRAIL-induced growth suppression/apoptosis of TRAIL-resistant colon carcinoma cell line RKO. Real-time cell proliferation assay (xCELLigence, Roche) and phase-contrast photographs.

- AS CR, KAN200520703 – The use of ultrasound in nanomedicine, 2007-2011, L. Anděra
- AS CR, KJB500520801 – Dissection of Bid function in TRAIL-induced apoptosis, 2008-2010, M. Koc
- Ministry of Health of the Czech Republic, NS10287 – Experimental therapy of mantle cell lymphoma (MCL), 2009-2011, L. Anděra
- GA CR, GAP301/10/1971 – Expression, signaling and function of Death Receptors in human embryonic stem cells, 2010-2012, L. Anděra
- FP6 EU, 37278 ONCODEATH - Sensitisation and resistant determinants of cancer cells to death receptor related therapies, 2006-2010, L. Anděra
- Ministry of Education, Youth and Sports of the Czech Republic, 1M0506 – Centre of Molecular and Cellular Immunology, 2005-2011, V. Hořejší, L. Anděra

1. Zivny J, Klener P Jr, Pytlik R, Andera L. The role of apoptosis in cancer development and treatment: focusing on the development and treatment of hematologic malignancies. **Curr Pharm Des 2010** 16(1):11-33.
2. Oikonomou E, Kosmidou V, Katseli A, Kothonidis K, Mourtzoukou D, Kontogeorgos G, Andera L, Zografos G, Pintzas A. TRAIL receptor upregulation and the implication of KRAS/BRAF mutations in human colon cancer tumors. **Int J Cancer 2009** 125(9): 2127-2135.
3. Klíma M, Zájedová J, Doubravská L, Anděra L. Functional analysis of the posttranslational modifications of the death receptor 6. **Biochim Biophys Acta 2009** 1793(10): 1579-1587.
4. Doubravská L, Šímová Š, Cermak L, Valenta T, Kořínek V, Anděra L. Wnt-expressing rat embryonic fibroblasts suppress Apo2L/TRAIL-induced apoptosis of human leukemia cells. **Apoptosis 2008** 13(4): 573-587.
5. Šímová Š, Klíma M, Cermak L, Šourková V, Anděra L. Arf and Rho GAP adapter protein ARAP1 participates in the mobilization of TRAIL-R1/DR4 to the plasma membrane. **Apoptosis 2008** 13(3): 423-436.

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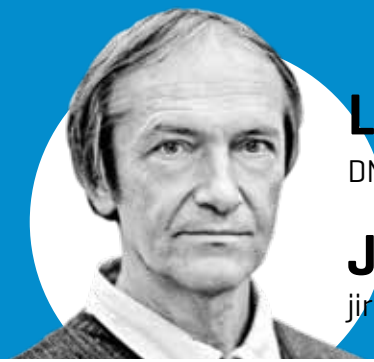

Laboratory of Genome Integrity

DNA damage response, cell cycle, oncogenic transformation, cellular senescence, ageing

Jiří Bartek

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Realization of complex tasks of living organisms depends on information stored in DNA of their genomes. The loss of this information due to endogenous and exogenous physicochemical damage of DNA results in disintegration of homeostasis at the cellular and organism level manifested as diseases including cancer and ageing. Several tightly orchestrated mechanisms take care to preserve the intactness of genetic information by preventing and repairing DNA damage. Our research is centered on cellular responses (termed collectively DNA damage response; DDR) to DNA double-strand breaks, presumably the most deleterious lesions affecting DNA. Cells with unhealed chromosomal breaks are mostly prevented from cell division due to activated DNA damage cell cycle checkpoints; however, following unscheduled cell division, unrepaired breaks result in chromosomal instability with accompanying changes in gene dosage – the driving force of malignant transformation. Specifically we focus on 1) posttranslational modifications (phosphorylation, ubiquitylation, sumoylation and acetylation) of key players involved in sensing and transmitting signals from DNA breaks to cellular effectors responsible for activation of cell cycle checkpoints and repair; 2) mechanisms of DNA damage cell cycle checkpoint recovery after successful DNA repair; 3) mechanisms of cellular response to chronic irreparable DNA damage manifested as irreversible cell cycle arrest (cellular senescence); and 4) role of DNA damage-activated expression of secreted factors (cytokines) in autocrine/paracrine signalling and intercellular communication, and 5) impact of the above mechanisms on cancer and ageing.

Recently, we have characterized cytokine expression in chemotherapeutic drug-induced cellular senescence and the role of activated interferon JAK/STAT signalling pathway in autocrine/paracrine induction of tumour suppressors such as PML, STAT1 and IRF-1. We have found that normal and tumour human cells escaping acute intoxication with specific bacterial toxin, cytolethal distending toxin, undergo irreversible cell cycle arrest with all hallmarks of cellular senescence including irreparable chromosomal damage and cytokine expression.

In collaboration with R. Medema group (University of Utrecht, Netherlands), we have investigated the role of Wip1 phosphatase in dephosphorylation of DNA damage-associated modification of histone protein H2AX and in DNA damage checkpoint recovery. Currently, we are performing high-throughput siRNA-based phenotypic screening to discover factors involved in posttranslational modifications of DDR components and functional characterization of positive 'hits'.

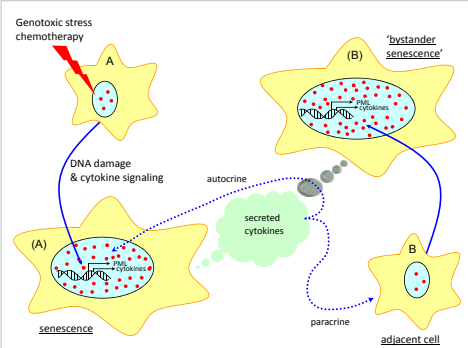


Fig. 1. Model of PML and cytokine signalling in drug-induced and 'bystander' senescence. Cytokine secretion and autocrine/paracrine signalling triggered by the DDR machinery upregulate PML expression and formation of PML NBs, collectively leading to cellular senescence, both directly and through 'bystander' effects.

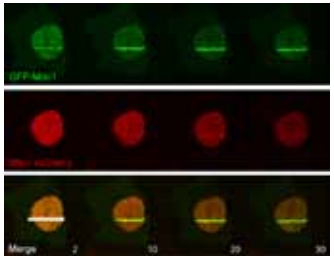
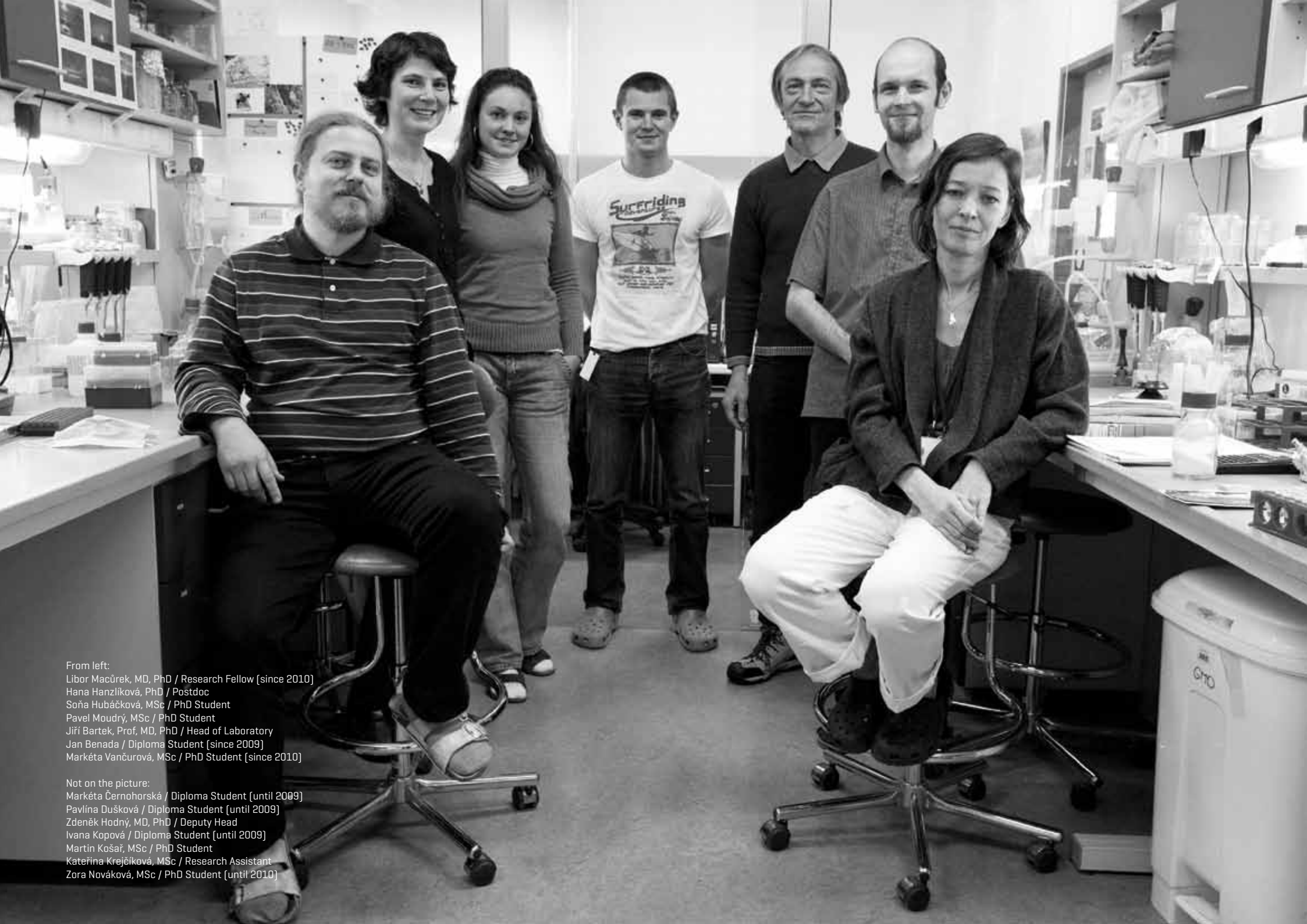


Fig. 2. Wip1 phosphatase translocates to sites of DNA damage. U2OS cells transfected with MDC1-GFP and Wip1-mCherry plasmids were grown in glass-bottom wells in a permanently heated chamber and were presensitized by Hoechst-33342 (0.5 μg/ml) treatment for 1 h. DNA damage was induced by microirradiation of indicated regions with a 405 nm laser. Images were acquired at the indicated times after microirradiation (min).

- AS CR, IAA500390501 – The role of PML in cellular senescence, 2005-2009, Z. Hodný
- GA CR, GA301/08/0353 – Protein modifications in DNA damage signalling: Mechanisms and cancer relevance, 2008-2010, J. Bartek
- GA CR, GA204/08/1418 – The role of the JAK/STAT signalling pathway in cellular senescence, 2008-2012, Z. Hodný
- FP7 EU, 223575 TRIREME – Systems-level, multi-layer understanding of cellular responses to ionizing radiation, 2009-2012, J. Bartek
- GA CR, GAP301/10/1525 – Mechanisms of DNA damage checkpoint termination, 2010-2012, J. Bartek
- GA CR, GPP305/10/P420 – Role of Wip1 phosphatase in the DNA damage response, 2010-2012, L. Macůrek

1. Hubackova S, Novakova Z, Krejcikova K, Kosar M, Dobrovolna J, Duskova P, Hanzlikova H, Vancurova M, Barath P, Bartek J, Hodny Z. Regulation of the PML tumor suppressor in drug-induced senescence of human normal and cancer cells by JAK/STAT-mediated signaling. **Cell Cycle 2010** 9(15): 3085-3099.
2. Bartkova J, Moudry P, Hodny Z, Lukas J, Rajpert-De Meyts E, Bartek J. Heterochromatin marks HP1γ, HP1α and H3K9me3, and DNA damage response activation in human testis development and germ cell tumours. **Int J Androl 2010** Published online: 1 AUG 2010, DOI: 10.1111/j.1365-2605.2010.01096.x.
3. Macůrek L, Lindqvist A, Voets O, Kool J, Vos HR, Medema RH. Wip1 phosphatase is associated with chromatin and dephosphorylates γH2AX to promote checkpoint inhibition. **Oncogene 2010** 29(15): 2281-2291.
4. Novakova Z, Hubackova S, Kosar M, Janderova-Rossmeislova L, Dobrovolna J, Vasicova P, Vancurova M, Horejsi Z, Hozak P, Bartek J, Hodny Z. Cytokine expression and signaling in drug-induced cellular senescence. **Oncogene 2010** 29(2): 273-284.
5. Blazkova H, Krejcikova K, Moudry P, Frisan T, Hodny Z, Bartek J. Bacterial intoxication evokes cellular senescence with persistent DNA damage and cytokine signaling. **J Cell Mol Med 2010** 14(1-2): 357-367.



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Martin Košař, MSc / PhD Student
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Laboratory of Cell Differentiation

Chemical genetics, haematopoietic and neural cell differentiation, signalling pathways, nuclear receptors

Petr Bartůněk

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The main interest of the laboratory lies in the molecular mechanism of cell fate determination. In the lab we have established *in vitro* systems to study the self-renewal and differentiation of haematopoietic, neural and mesenchymal stem cells. We use growth factors and small molecules as tools to manipulate these systems. More recently, we have initiated more systematic search for such tools using chemical biology/genetics approaches.

Nuclear receptors function as ligand-dependent transcription factors to regulate gene transcription in response to specific physiological stimuli such as steroids, retinoids, thyroid hormone and vitamin D. Thyroid hormone receptors, activated in response to thyroid hormone (T3), are known to modulate the level of serum cholesterol via complex regulatory pathways. By screening for T3-regulated genes we have identified Disp3, a sterol-sensing domain-containing protein that is related to the Dispatched family of proteins. DISP3 is predominantly expressed in specific cell types of the brain, retina and testis and localizes within the endoplasmic reticulum, and was found to co-localize with cholesterol. Ectopic expression of DISP3 in fibroblasts resulted in elevated cholesterol levels combined with an altered cholesterol distribution. We proposed that DISP3 represents a new molecular link between thyroid hormone and cholesterol metabolism in the brain
(Zikova *et al.* 2009). Moreover, we have identified two neural stem cell lines that highly express Disp3. Disp3 expression is positively regulated by T3 treatment, and upon differentiation the level of Disp3 dramatically changes, suggesting that Disp3 may modulate self-renewal or differentiation. Brain tumours such as medulloblastoma are believed to arise from neural precursor cells. Analysis of a small number of primary human tumours revealed very high expression of Disp3, suggesting an important role for this protein in their pathogenesis. We have performed RNAi and overexpression studies and found out that Disp3 is able to modulate the cell fate of neural stem cells and their progeny.

We have also identified, cloned and characterized the first non-mammalian Tpo, chicken thrombopoietin, and its receptor c-Mpl. Discovery of chicken Tpo and c-Mpl will greatly facilitate future studies regarding thrombocytic differentiation and haematopoietic stem cell development. Moreover, we have introduced an experimental model of chicken bi-potent thrombo/erythropoietic progenitors that can be used to identify key regulators of cell fate determination (Bartunek *et al.* 2008). In addition, we have extended our studies to vertebrate hematopoietic development by introducing a new model organism in our laboratory – the zebrafish – and we established the first *ex vivo* cultures of haematopoitic cells (Stachura *et al.* 2009).

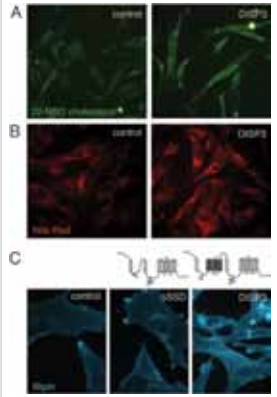

Fig. 1. Ectopic expression of DISP3 leads to accumulation of lipid droplets. (A) Accumulation of exogenous NBD-cholesterol in lipid droplets in control and DISP3-expressing cells, (B) Control and DISP3 cells stained by Nile Red to evaluate lipid droplet formation. Note the increased number of lipid droplets in DISP3-expressing cells. (C) Cholesterol accumulation and relocalization is compromised in DSSD-DISP3-expressing cells. CEF cells stably transfected with either an empty vector, vector encoding DSSD-DISP3, or wt DISP3 were analysed by filipin staining.

Fig. 2. Zebrafish as a model to study vertebrate haematopoiesis. Doublehemizygous transgenic zebrafish Tg(gata1::DsRed); Tg(mpx::EGFP) at 4 days post fertilization with single haematopoietic cells fluorescently labelled (red, erythroid cells, green, myeloid cells/neutrophils).

- Ministry of Education, Youth and Sports of the Czech Republic, LC06077 – Centre of Chemical Genetics, 2006-2010, P. Bartůněk
- FP6 EU, 18652 CRESCENDO – Consortium for Research into Nuclear Receptors in Development and Aging, 2006-2011, P. Bartůněk
- FP7 EU, 261861 EU-OPENSCREEN – European Infrastructure of Open Screening Platforms for Chemical Biology, 2010-2013, P. Bartůněk
- AS CR, IAA500520705 – Characterization of the new member of sterol-sensing-domain protein family and its role in lipid metabolism, 2007-2009, M. Zíková
- GA CR, GA310/08/0878 – The role of the cells prion protein in erytroidnic differentiation: possible link to peripheral pathogenesis of prion diseases, 2008-2012, P. Bartůněk
- GA CR, GA204/09/1905 – Disp3: A potential role in the self renewal and differentiation of neural stem cells, 2009-2012, P. Bartůněk
- GA CR, GAP305/10/0953 – New regulators of megakaryocyte and erythroid lineage commitment, 2010-2013, P. Bartůněk
- Ministry of Industry and Trade, FT-TA5/136 – System for identification of biologically active compounds: Focused combinatorial libraries, P. Bartůněk

1. Eignerová B, Sedlák D, Dračínský M, Bartůněk P, Kotora M. Synthesis and Biochemical Characterization of a Series of 17alpha-Perfluoroalkylated Estradiols as Selective Ligands for Estrogen Receptor alpha. **J Med Chem 2010** 53(19): 6947-6953.
2. Sedlák D, Novák P, Kotora M, Bartůněk P. Synthesis and evaluation of 17α-arylestradiols as ligands for estrogen receptor α and β. **J Med Chem** 2010 53(10): 4290-4294.
3. Stachura DL, Reyes JR, Bartunek P, Paw BH, Zon LI, Traver D. Zebrafish kidney stromal cell lines support multilineage hematopoiesis. **Blood 2009** 114(2): 279-289.
4. Zikova M, Corlett A, Bendova Z, Pajer P, Bartunek P. DISP3, a sterol-sensing domain-containing protein that links thyroid hormone action and cholesterol metabolism. **Mol Endocrinol 2009** 23(4): 520-528.
5. Bartunek P, Karafiat V, Bartunkova J, Pajer P, Dvorakova M, Kralova J, Zenke M, Dvorak M. Impact of chicken thrombopoietin and its receptor c-Mpl on hematopoietic cell development. **Exp Hematol 2008** 36(4): 495-505.

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Laboratory of Molecular Pharmacology

G protein-coupled receptors, GPCR, neurotransmitter, glutamate signalling, cannabinoid signalling

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In mammalian brain the major excitatory neurotransmitter glutamate activates two types of receptors: ligand-operated ion channels (NMDA, AMPA and kainate receptors) and G protein-coupled receptors (GPCRs). There are eight genes that code for the metabotropic glutamate (mGlu) receptors in mammals. These receptors diverge in location within brain regions and cellular compartments and have distinct functions. As such, they constitute possible promising targets for treatment of several neurological diseases. Our research is focused on the structure-function relationships of these receptors and molecular machinery that regulates their signalling properties. The mGlu receptors belong to family C GPCRs and are traditionally viewed as composed of two identical subunits. Using the mutagenesis approach combined with a functional expression system we showed that within their homodimeric complexes only one HD reaches active state. Our recent data using dynamic FRET approach are in accord with this notion. The activation process of these family 3 GPCRs is thus asymmetrical. Recent data suggest that the mGluRs can also form heterodimers. We published data suggesting that heterodimerization between distinct splice variants of the mGlu1 receptor, the mGluR1a and mGluR1b, results in novel receptor complexes with altered function and trafficking properties in tranfected heterologous cells. Now we aim at revealing the situation in brain using a set of splice variant-specific antibodies we developed.

The cannabinoid receptor project is focused on regulation of signalling of the CB1 receptor 1. It is approached by molecular biology techniques combined with biochemical tools including yeast two-hybrid screen. Currently, several hits obtained by the latter technique are being analysed. One highly promising lead is investigated in deep detail. The mechanism of regulation of cell-surface stability of the CB1 receptor by endocytosis machinery proteins shows possible novel player(s). The mechanism of internalization of the CB1 receptor and its regulation is therefore currently under heavy investigation in our lab.

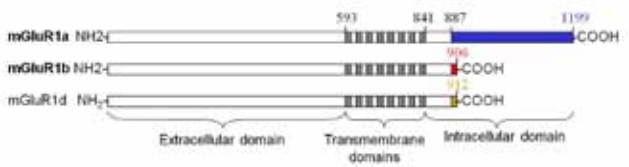

Fig. 1. Splicing of rat metabotropic glutamate receptor 1 (mGluR1) gene results in expression of long and short forms. Following the heptahelical domain and short sequence including RRKK motive (Endoplasmic Reticulum retention signal), the long form mGluR1a has a unique sequence of 312 aa, short forms are termed mGluR1b and d. The mGluR1b unique sequence is 19 aa long following the splicing site.

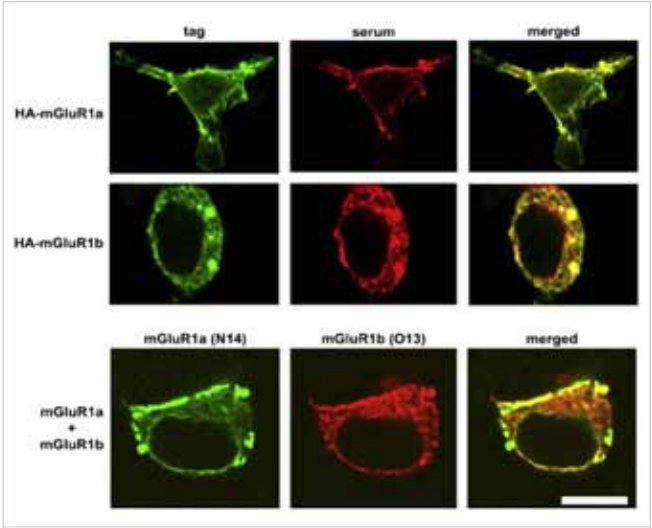

Fig. 2. For immunocytochemistry cells were transfected with HA-mGluR1a and stained with monoclonal anti-HA antibodies (secondary antibodies labelled with FITC) and our N14 antibodies (secondary anti-rabbit antibodies labelled with Cy3). c-Myc mGluR1b-expressing cells were labelled with anti-c-Myc antibodies and guinea pig anti-mGluR1b antibodies (O13) and detected with secondary antibodies (FITC, Cy3, respectively). Their patterns confirm specificity of the novel antibodies by overlap of corresponding anti-tag antibodies and staining with the subunit-specific sera. Bar equals to 10 μm *in vivo*.

- AS CR, IAA500390701 – Role of proteins associated with Cannabinoid receptor CB1 in trafficking, 2007-2011, J. Blahoš
- GA CR, GA303/08/1591 – Study of glutamate receptors conformational changes using novel fluorescent techniques, 2008-2012, J. Blahoš
- Ministry of Education, Youth and Sports of the Czech Republic, LC06063 – Fluorescence Microscopy in Biological and Medical Research, 2006-2011, P. Hozák, J. Blahoš

1. Pavlowsky A, Gianfelice A, Pallotto M, Zanchi A, Vara H, Khelfaoui M, Valnegri P, Rezai X, Bassani S, Brambilla D, Kumpost J, Blahos J, Roux MJ, Humeau Y, Chelly J, Passafaro M, Giustetto M, Billuart P, Sala C. A postsynaptic signaling pathway that may account for the cognitive defect due to IL1RAPL1 mutation. **Curr Biol 2010** 20(2): 103-115.
2. Pavek P, Pospechova K, Svecova L, Syrova Z, Stejskalova L, Blazkova J, Dvorak Z, Blahos J. Intestinal cell-specific vitamin D receptor (VDR)-mediated transcriptional regulation of CYP3A4 gene. **Biochem Pharmacol 2010** 79(2): 277-287.
3. Iacovelli L, Molinaro G, Battaglia G, Motolese M, Di Menna L, Alfiero M, Blahos J, Matrisciano F, Corsi M, Corti C, Bruno V, De Blasi A, Nicoletti F. Regulation of group II metabotropic glutamate receptors by G protein-coupled receptor kinases: mGlu2 receptors are resistant to homologous desensitization. **Mol Pharmacol 2009** 75(4): 991-1003.
4. Kumpost J, Syrova Z, Kulihova L, Frankova D, Bologna JC, Hlavackova V, Prezeau L, Kralikova M, Hruskova B, Pin JP, Blahos J. Surface expression of metabotropic glutamate receptor variants mGluR1a and mGluR1b in transfected HEK293 cells. **Neuropharmacology 2008** 55(4): 409-418.

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Laboratory of Biology of Cytoskeleton

Modulation of microtubule organization, microtubule proteins, signal transduction

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The long-term research programme of the laboratory has been focused on studying the structure-function relationships of microtubule (MT) proteins and their interactions with other cytoskeletal elements in cells under normal and pathological conditions. The organization of dynamic MT networks is controlled by microtubule organizing centres (MTOCs). One of the key components of MTOCs is γ-tubulin, which is necessary for nucleation of MT. Current work focuses on the understanding of the modulation of MT properties by signal transduction molecules, the function of γ-tubulin forms, and molecular and functional characterization of regulators of microtubule nucleation. To address these questions, techniques of molecular biology, biochemistry and immunology are being used, as well as a variety of microscopic techniques, including live cell imaging using TIRF microscopy. Our results demonstrate that rearrangement of microtubules in activated mast cells depends on activity of the stromal interacting protein 1 (STIM1) and that Ca^{2+} plays an important role in the formation of microtubule protrusions in activated cells. We have also shown that non-receptor protein tyrosine kinases play an important role in MT nucleation. We have demonstrated that prediction of epitope exposure on microtubules by means of homology modelling combined with site-directed antibodies can contribute to better understanding of the interactions of microtubules with associated proteins. Finally, we have shown that ectopic expression of tubulins may represent a novel marker in pathobiology of glioblastoma multiforme, the most common and deadliest form of primary brain cancers.

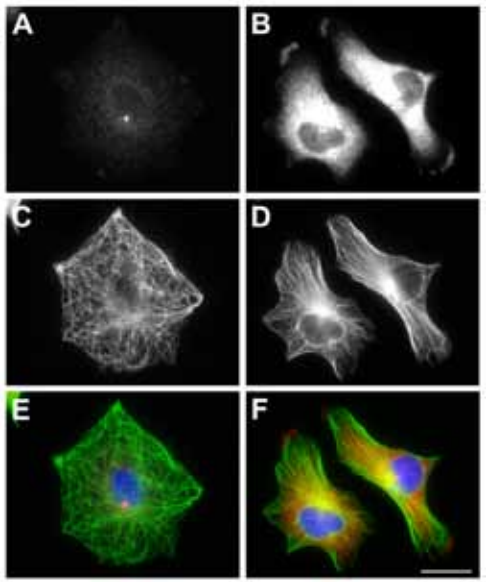

Fig. 1. Different subcellular distribution of γ-tubulin in fibroblasts and glioblastoma cells. Mouse fibroblasts 3T3 (A, C, E) or human glioblastoma cells T98G (B, D, F) stained with antibodies for γ-tubulin (A, B, red) and α-tubulin (C, D, green). Superpositions of images are shown in E and F. Scale bar, 20 μm.

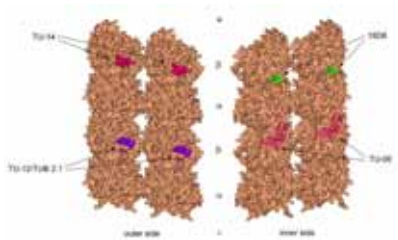

Fig. 2. Homology modelling of microtubule protofilaments with denoted positions of β-tubulin epitopes. Amino acids exposed on the surface of protofilaments are coloured for epitopes recognized by monoclonal antibodies. Outer and inner sides of adjacent protofilaments are depicted. α and β denote positions of tubulin subunits in protofilaments; (+) and (-) mark the orientation of protofilaments.

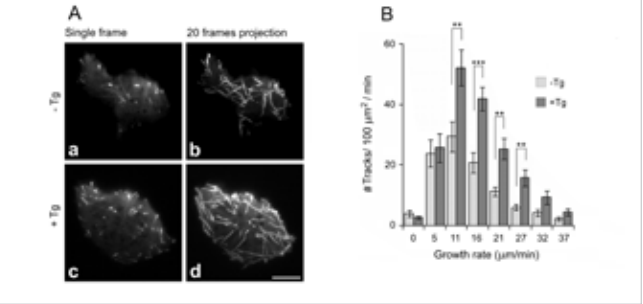

Fig. 3. Activation of mast cells increases the number of growing microtubules in cell periphery as determined by TIRFM time-lapse imaging. (A) Time-lapse imaging of resting (a-b) and thapsigargin (Tg)-activated (c-d) mast cells expressing EB1-GFP that marks growing microtubules. Still images of EB1 (a, c) and tracks of EB1 comets over 20 sec (b, d) in TIRFM. (B) Histogram of microtubule growth rates in cell periphery of resting (-Tg) and thapsigargin-activated (+Tg) cells.

- GA CR, GA204/09/1777 – Analysis of signalling assemblies containing γ-tubulin, 2009-2011, Pavel Dráber
- GA CR, GAP302/10/1701 – Analysis of functional differences between γ-tubulins, 2010-2012, E. Dráberová
- Ministry of Education, Youth and Sports of the Czech Republic, LC545 – Centre of Functional Organization of the Cell, 2005-2011, Pavel Dráber
- AS CR, KAN200520701 – Nano-PCR – ultrasensitive test for detection of specific proteins in body fluids, 2007-2011, Pavel Dráber

1. Hájková Z, Bugajev V, Dráberová E, Vinopal S, Dráberová L, Janáček J, Dráber Pe, Dráber P. STIM1-directed reorganization of microtubules in activated mast cells**. J Immunol** 2011 186(2): 913-923.
2. Dráberová E, Sulimenko V, Sulimenko T, Böhm KJ, Dráber P. Recovery of tubulin functions after freeze-drying in the presence of trehalose. **Anal Biochem 2010** 397(1): 67-72.
3. Blume Y, Yemets A, Sheremet Y, Nyporko A, Sulimenko V, Sulimenko T, Dráber P. Exposure of β-tubulin regions defined by antibodies on an *Arabidopsis thaliana* microtubule protofilament model and in the cells. **BMC Plant Biol 2010** 10(1): 29.
4. Katsetos CD, Dráberová E, Legido A, Dráber P. Tubulin targets in the pathobiology and therapy of glioblastoma multiforme. II. γ-Tubulin. **J Cell Physiol 2009** 221(3): 514-520.
5. Macurek L, Dráberová E, Richterová V, Sulimenko V, Sulimenko T, Dráberová L, Marková V, Dráber P. Regulation of microtubule nucleation from membranes by complexes of membrane-bound γ-tubulin with Fyn kinase and phosphoinositide 3-kinase. **Biochem J 2008** 416(3): 421-430.

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Laboratory of Signal Transduction

Plasma membrane signalosomes, mast cell, IgE receptor signalling

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The plasma membrane possesses a variety of molecules (mainly proteins and lipids) that are involved in such important cellular functions as cell migration, adhesion and signalling. Our recent studies have been focused on understanding the roles of selected plasma membrane-associated components at initial stages of mast cell activation. We analyzed functions of the transmembrane adaptor protein NTAL and the roles of protein tyrosine phosphatases (PTPs) at early stages of mast cell signalling induced via the high-affinity IgE receptor (FcεRI) or c-kit. We found that mast cells isolated from NTAL-deficient cells exhibited reduced spreading on fibronectin, enhanced filamentous actin depolymerization and enhanced migration towards antigen when compared to wild-type cells. To understand the molecular basis of these phenomena, we examined activities of two small GTPases, Rac and Rho, important regulators of actin polymerization. Stimulation of the cells via FcεRI enhanced activity of Rac[1,2,3] in both NTAL-deficient and wild-type cells. In contrast, the RhoA activity decreased and this trend was much faster and more extensive in NTAL-deficient cells, indicating a positive regulatory role of NTAL in the recovery of RhoA activity. After restoring NTAL into NTAL-deficient cells, both spreading and actin responses were rescued. Thus, our studies showed for the first time that NTAL has a crucial role in signalling, via RhoA, to the mast cell cytoskeleton.

The earliest known biochemical step in FcεRI-activated cells is tyrosine phosphorylation of the receptor subunits by Src family kinase Lyn. However, the exact molecular mechanism of this phosphorylation step is incompletely understood. We therefore tested our hypothesis that changes in the activity and/or topography of protein tyrosine phosphatases could play a major role in the FcεRI triggering. We found that exposure of mast cells to PTP inhibitors induced phosphorylation of the FcεRI subunits, similarly as FcεRI triggering. Interestingly, and in sharp contrast to antigen-induced activation, the inhibitors had no effect on association of FcεRI with detergent-resistant membranes and their topography in the plasma membrane. In cells stimulated with antigen or the inhibitors, enhanced oxidation of active site cysteine of several PTPs was detected. Unexpectedly, most of oxidized phosphatases bound to the plasma membrane were associated with the actin cytoskeleton. Based on these and other data we proposed that down-regulation of enzymatic activity of PTPs and/or changes in their accessibility to the substrates play a key role in initial tyrosine phosphorylation of the FcεRI and other multichain immune receptors.

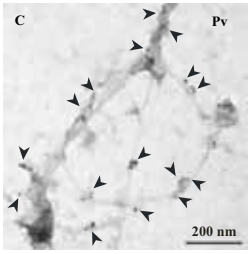

Fig. 1. Membrane topography of oxidized PTPs as detected by electron microscopy on isolated plasma membrane sheets (see Heneberg *et al.*, JBC 2010).

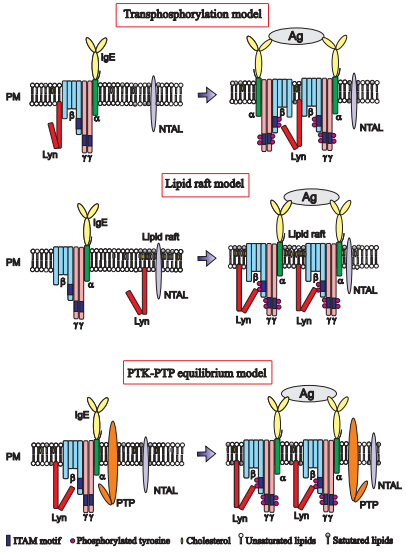

Fig. 2. Models of the high-affinity IgE receptor (FcεRI) phosphorylation by Lyn kinase (see Bugajev *et al.*, FEBS Lett 2010).

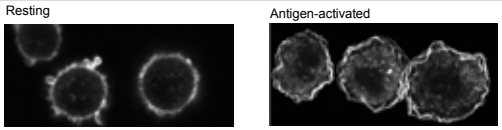

Fig. 3. Spreading of mast cells on fibronectin after antigen-induced activation (see Tůmová *et al.*, Eur J Immunol 2010).

- AS CR, KAN200520701 – Nano-PCR – ultrasensitive test for detection of specific proteins in body fluids, 2007-2011, Petr Dráber
- AS CR, M200520901 – Novel components of mast cell immune receptor signalling, 2009-2012, Petr Dráber
- GA CR, GA301/09/1826 – Topography and function of Csk-binding proteins of the plasma membrane in mast cells, 2009-2013, Petr Dráber
- GA CR, GP302/10/1759 – Function-structure relationships between transmembrane adaptor-based signalosomes in mast cells, 2010-2013, L. Dráberová

1. Heneberg P, Dráberová L, Bambousková M, Pompach P, Dráber P. Down-regulation of protein-tyrosine phosphatases activates an immune receptor in the absence of its translocation into lipid rafts. **J Biol Chem 2010** 285(17): 12787-12802.
2. Tůmová M, Koffer A, Šimíček M, Dráberová L, Dráber, P. The transmembrane adaptor protein NTAL signals to mast cell cytoskeleton via the small GTPase Rho. **Eur J Immunol 2010** 40(11): 3235-45.
3. Shaik GM, Dráberová L, Heneberg P, Dráber P. Vacuolin-1-modulated exocytosis and cell resealing in mast cells. **Cell Signal 2009** 21(8): 1337-1345.
4. Shaik GM, Dráberová L, Dráber P, Boubelík M, Dráber P. Tetraalkylammonium derivatives as real-time PCR enhancers and stabilizers of the qPCR mixtures containing SYBR Green I. **Nucleic Acids Res 2008** 36(15): e93.
5. Smrž D, Lebduška P, Dráberová L, Korb J, Dráber P. Engagement of phospholipid scramblase 1 in activated cells: Implication for phosphatidylserine externalization and exocytosis. **J Biol Chem 2008** 283(16): 10904-10918.

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Laboratory of Molecular Virology

Carcinogenesis, cell differentiation, photodynamic therapy

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The recent research efforts of the group focus on genes and molecular mechanisms involved in 1) malignant transformation of haematopoietic cells, melanocytes, nephrogenic blastema, liver and lung cells; 2) metastasis and interactions between a tumour and its microenvironment; 3) fate determination in multipotent neural cells and differentiation of myogenic precursors; 4) apoptosis induced by photoactivation of specific porphyrins and development of porphyrin derivatives for potential use in photodynamic therapy. In studies on cell fate determination, differentiation and malignant transformation of haematopoietic and neural cells (collaboration with the Institute of Anatomy, Prague), *myb* genes are used as tools to modulate development of avian cells and tissues. In studies on genes involved in the formation of kidney, liver and lung tumours in chicks, insertional mutagenesis by MAV retroviruses is exploited. Genes of the *egr* family serve as tools to affect epithelial and mesenchymal cell phenotypes and metastatic potential of experimental tumours. Porphyrin derivatives synthesized by the cooperating group (Institute of Chemical Technology, Prague) are used for experiments with targeted drug delivery and induction of cell death in cancer cells and tissues.

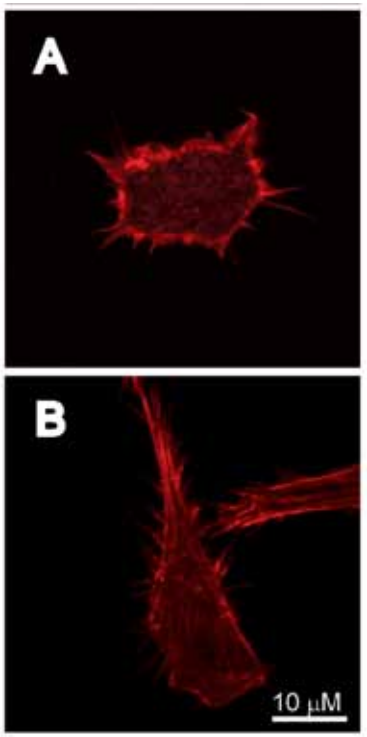


Fig. 1. Induction of the metastatic potential of the experimental tumour. The non-metastatic tumour cell (A) changes its phenotype (B) and acquires metastatic potential following Egr1 expression.

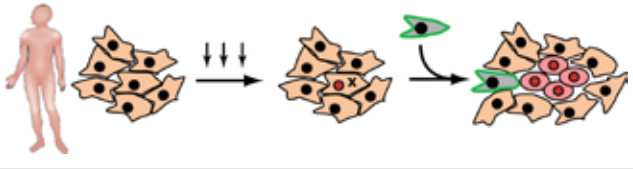


Fig. 2. The scheme of industasis – a previously unreported mechanism of promotion of malignant tumours. Tumour promotion has been very likely the trigger of the majority of human tumours. Tumour promotion consists of events capable of waking up a dormant incipient tumour cell (depicted by the cross) which has already accumulated genetic mutations (arrows) but remains under control of the surrounding microenvironment. It was found that normal non-malignant stray cells (green cell) can function as a tumour-promoting stimulus. We hypothesize that industasis might be the underlying cause of human multiple primary tumours.

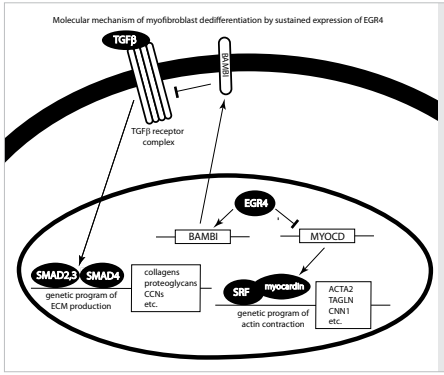
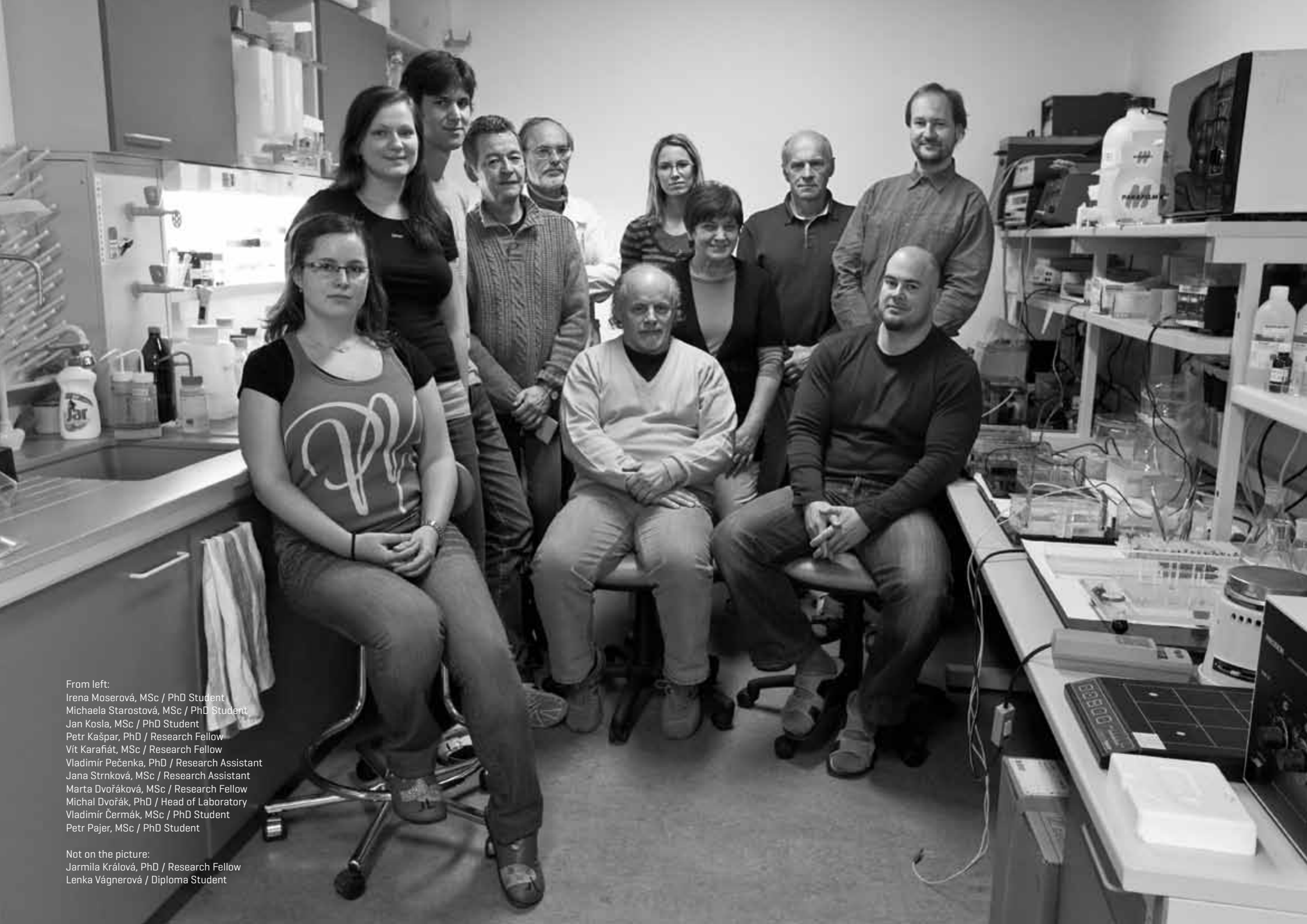


Fig. 3. Egr4 mechanism: The proposed mechanism of myofibroblast dedifferentiation by Egr4. We found that Egr4 both activates synthesis of BAMBI, the negative regulator of TGFβ signal, and blocks expression of myocardin, the gene necessary for the function of the cell contraction apparatus. In this way can Egr4 suppress those characteristics of myofibroblasts that support the onset of fibrosis.

- Ministry of Education, Youth and Sports of the Czech Republic, LC06061 – Centre of Cell Invasiveness in Embryonic Development and Tumour Metastases, 2006-2011, M. Dvořák
- AS CR, KAN200200651 – Nanoparticulate and supramolecular systems for targeted drug delivery, 2006-2010, J. Králová
- GA CR, GA301/09/1727 – Large-scale identification of genes responsible for the formation of solid tumours, 2009-2012, M. Dvořák
- GA CR, GA203/09/1311 – Synthetic probes for recognition of tumour markers: applications for cell directed apoptosis and targeted photodynamic therapy, 2009-2012, J. Králová
- GA CR, GAP305/10/2133 – The study of satellite cells migration, 2010-2012, P. Kašpar

1. Čermák V, Kosla J, Plachý J, Trejbalová K, Hejnar J, Dvořák M. The transcription factor EGR1 regulates metastatic potential of v-src transformed sarcoma cells. **Cell Mol Life Sci 2010** 67(20): 3557-3568.
2. Králová J, Kejík Z, Bříza T, Poučková P, Král A, Martásek P, Král V. Porphyrin-cyclodextrin conjugates as a nanosystem for versatile drug delivery and multimodal cancer therapy. **J Med Chem 2010** 53(1): 128-138.
3. Pajer P, Karafiát V, Pečenka V, Průková D, Dudlová J, Plachý J, Kašparová P, Dvořák M. Industasis, a promotion of tumor formation by nontumorigenic stray cells. **Cancer Res 2009** 69(11): 4605-4612.
4. Kralova J, Dvorak M, Koc M, Kral V. p38 MAPK plays an essential role in apoptosis induced by photoactivation of a novel ethylene glycol porphyrin derivative. **Oncogene 2008** 27(21): 3010-3020.
5. Kašpar P, Dvořák M. Involvement of phosphatidylserine externalization in the down-regulation of c-myb expression in differentiating C2C12 cells. **Differentiation 2008** 76(3): 245-252.



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Laboratory of Immunobiology

Innate immune receptors, neutrophils, defensins, TCR signalling

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The group was established in the summer of 2007. The central theme of our research is the molecular and functional characterization of innate immune mechanisms involved in the process of sterile inflammation, developmental tissue remodelling and chronic inflammatory diseases. Our main effort is focused on Toll-like receptors and other TIR domain-containing immune-related proteins and their role in the early embryogenesis. Data accumulated so far point to the spatially and temporarily regulated expression of TLRs on embryonal phagocytes, suggesting their involvement in sterile inflammation during early development. The characterization of a complete set of innate immune receptors (IIRs) expressed on embryonal phagocytes and evaluation of their signalling competence, together with elucidation of the nature of endogenous ligands for these IIRs represent our main objectives. Further, the cDNA microarray analyses performed on embryonal phagocytes revealed cell-specific expression of several uncharacterized molecules that could play an essential role in the processes supporting embryonal homeostasis. In collaboration with our partner laboratories we have also characterized immunomodulatory activities of enzymatically synthesized oligofuranosides (1). Recent data indicate their robust adjuvant properties with prophylactic and therapeutic potential. Our research is also geared towards the understanding of the contribution of cellular and humoral innate immune factors to the onset and maintenance of autoimmune processes. In collaboration with clinical laboratories we were able to demonstrate increased levels of defensin expression in recently diagnosed patients suffering from autoimmune diseases. Our main mission here is to characterize novel predictive biomarkers suitable for diagnosis of these diseases in the pre-clinical phase of their development. In this context we have also characterized the distribution and tissue-specific functions of alpha-defensins in rat experimental models (2). We also continue in our effort to understand very early biochemical events leading to activation of T cells. Our previous study showed that the critical event in this process is the translocation and subsequent enrichment of kinase active Lck in lipid rafts (LR) (3). While other regulatory proteins are also recruited to LR upon T-cell activation, the mechanism of these translocations, indispensable for T-cell activation, is largely unknown. The main goal of this line of research is the characterization of the molecular mechanism and its structural elements underpinning the recruitment of Lck and other signalling molecules to LR. In addition, we provide technical and knowledge support for exploration of several molecular approaches in unrelated projects (4, 5).

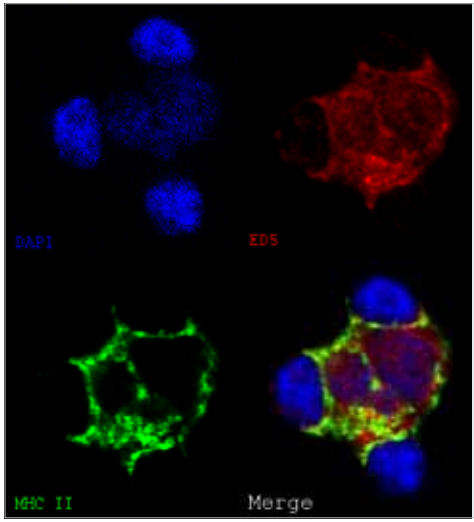

Fig. 2. Medullar thymic epithelial cells (mTECs) of Wistar rat produce enteric defensin ED5. Imunofluorescent confocal microscopy revealed multiple cellular interactions between mTEC and thymocytes.

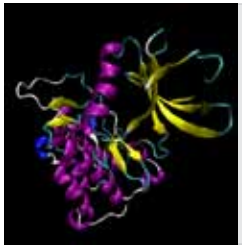

Fig. 1. Using a combination of biochemical, genetic and computer modelling approaches provides new insights into the regulation of Lck activity (image by V. Spiwok, MSc, ICT, Prague).

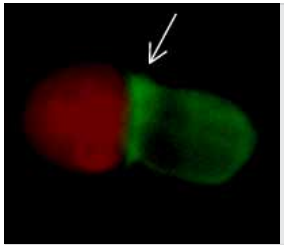

Fig. 3. Newly identified adaptor protein (green) co-redistribute with Lck into forming immunological synapse (arrow) during early phases of $CD4^+$ T-cell/APC interaction.

- AS CR, IAA500520707 – The role of innate immune molecules in embryonic homeostasis and sterile inflammation, 2007-2011, D. Filipp
- Ministry of Education, Youth and Sports of the Czech Republic, 2B08066 – Novel treatment of genetically determined metabolic disease, type 1 diabetes, using an immunotherapeutical approach, 2008-2011, D. Filipp
- Ministry of Defence of the Czech Republic, OVUOFVZ200808 - Development of new prophylactic tools against Francisella tularensis infection, 2008-2011, D. Filipp
- GA CR, GA310/09/2084 – Characterization of the molecular machinery regulating the recruitment of signalling molecules to lipid rafts, 2009-2013, D. Filipp

1. Chlubnová I, Filipp D, Spiwok V, Dvořáková H, Daniellou R, Nugier-Chauvin C, Králová B, Ferrieres V. Enzymatic synthesis of oligo-D-galactofuranosides and L-arabinofuranosides: from molecular dynamics to immunological assays. **Org Biomol Chem 2010** 8(9): 2092-2102.
2. Neuwirth A, Dobeš J, Cmarko D, Selsted ME, Filipp D. Unexpected complexity of alpha-defensin expression in the rat thymus (submitted).
3. Filipp D, Moemeni B, Ferzoco A, Kathirkamathamby K, Zhang J, Ballek O, Davidson D, Veillette A, Julius M. Lck-dependent Fyn activation requires C terminus-dependent targeting of kinase-active Lck to lipid rafts. **J Biol Chem 2008** 283(39): 26409-26422.
4. Jirsova K, Neuwirth A, Kalasova S, Vesela V, Merjava S. Mesothelial proteins are expressed in the human cornea. **Exp Eye Res 2010** 91(5): 623-629.
5. Merjavá S, Neuwirth A, Tanzerová M, Jirsová K. The spectrum of cytokeratins expressed in the adult human cornea, limbus and perilimbal conjunctiva. **Histol Histopathol 2011** 26(3): 323-331.

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Meiotic silencing, aneuploidy, genomics, hybrid sterility, *Prdm9*

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Genetic architecture of hybrid male sterility between *Mus m. musculus* and *Mus m. domesticus* subspecies has been analysed on the model of PWD/Ph x C57BL/6 sterile male hybrids. The candidate genes were evaluated by transgenic rescue for the *Hst1* locus and by positional cloning and expression profiling of sorted testicular cells for the *Hstx1* and *Hstx2* loci. We have identified the first vertebrate hybrid sterility locus, the mouse Hybrid sterity 1 (*Hst1*) with *Prdm9* (*Meisetz*), encoding a meiotic histone H3 lysine-4 tri-methyltransferase. Positional cloning was confirmed by hybrid male infertility rescue by using the intact *Prdm9* transgene in bacterial artificial chromosomes with the "fertility" *Hst1*^{f} allele. Identification of the first vertebrate hybrid sterility gene reveals a role for epigenetics in speciation, and opens a window to a hybrid sterility gene network. We have established a new mouse model of human aneuploidy syndromes. The Ts43H segmental trisomy of proximal 30 MB of mouse chromosome 17 encompasses over 300 protein-coding genes. Chromosome substitution strains C57BL/6.PWD, recently constructed in our laboratory, are used for phenome analysis in collaboration with The Jackson Laboratory, Bar Harbor, Maine, USA (Dr. K.L. Svenson) and for the genetics of gene expression and splicing in a systems genetics project with the Max-Planck-Institute for Molecular Genetics in Berlin (Prof. H. Lehrach). We study meiotic X-chromosome inactivation by genome-wide expression profiling and by monitoring transcription profiles and histone modifications in meiotic and postmeiotic testicular cells of carriers of male-sterile autosomal rearrangements and in male-sterile inter-species hybrids.

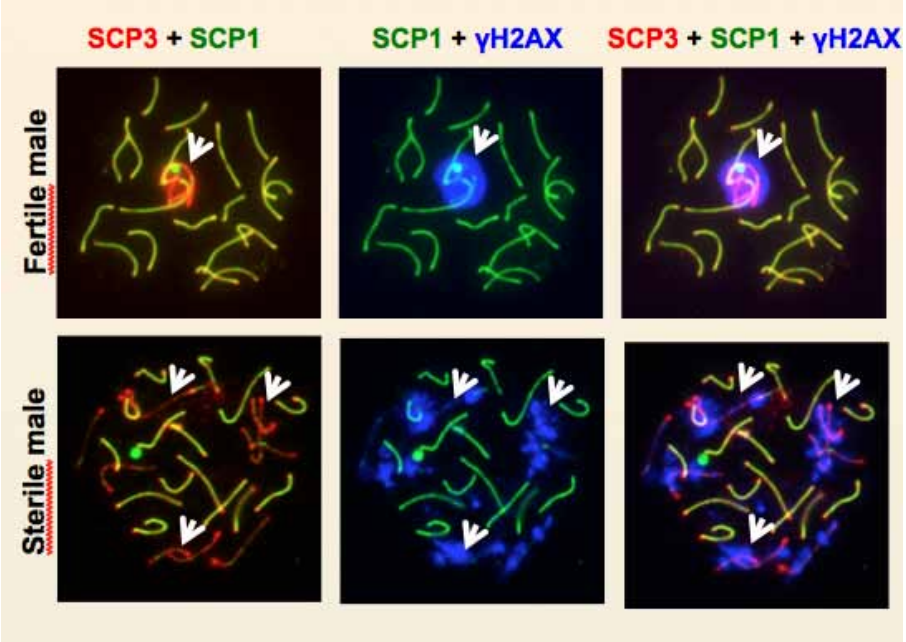

Fig. 1. Pachytene spermatocytes of sterile and fertile hybrids, (PWD x B6)F1 and (B6 x PWD)F1, display unsynapsed autosomes and dislocated histone γH2AX.

- GA CR, GA301/07/1383 – Gene expression profiling during mouse spermatogenesis: Implications for meiotic sex chromosome inactivation and gene retroduplication, 2007-2009, P. Jansa
- GA CR, GA301/07/1264 – Uncovering regulatory networks in novel mouse models by genetical genomics, 2007-2009, J. Forejt
- GA CR, GAP305/10/1931 – Identification of interactors of the meiotic histone methyltransferase Hybrid sterility 1 (PR-domain 9), 2010-2013, Z. Trachtulec
- AS CR – Premium Academiae, 2007-2013, J. Forejt
- Ministry of Education, Youth and Sports of the Czech Republic, 1M0520 – Center for Applied Genomics, 2005-2011, J. Forejt
- FP6 EU, 37627 ANEUPLOIDY - AnEUploidy: understanding gene dosage imbalance in human health using genetics, functional genomics and systems biology, 2006-2011, J. Forejt

1. Homolka D, Ivanek R, Forejt J, Jansa P. Differential expression of non-coding RNAs and continuous evolution of the X chromosome in testicular transcriptome of two mouse species. **PLoS ONE 2011** 6(2): e17198.
2. Mihola O, Trachtulec Z, Vlcek C, Schimenti JC, Forejt J. A mouse speciation gene encodes a meiotic histone H3 methyltransferase. **Science 2009** 323(5912): 373-375.
3. Piálek J, Vyskočilová M, Bímová B, Havelková D, Piálková J, Dufková P, Bencová V, Ďureje L, Albrecht T, Hauffe HC, Macholán M, Munclinger P, Storchová R, Zajícová A, Holáň V, Gregorová S, Forejt J. Development of unique house mouse resources suitable for evolutionary studies of speciation. **J Hered 2008** 99(1): 34-44.
4. Gregorová S, Divina P, Storchova R, Trachtulec Z, Fotopulosova V, Svenson KL, Donahue LR, Paigen B, Forejt J. Mouse consomic strains: exploiting genetic divergence between Mus m. musculus and Mus m. domesticus subspecies. **Genome Res 2008** 18(3): 509-515.
5. Trachtulec Z, Vlcek C, Mihola O, Gregorova S, Fotopulosova V, Forejt J. Fine haplotype structure of a chromosome 17 region in the laboratory and wild mouse. **Genetics 2008** 178(3): 1777-1784.

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Receptors for retroviruses, retroviral vectors, endogenous retroviruses, integration and silencing of retroviruses

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Retroviruses interact with the host cells at multiple levels. They enter cells via specific receptors, integrate into the host genome, and use the cell transcription machinery to express their structural or enzymatically active proteins. Specific binding of retroviral envelope proteins to host cell receptors is the prerequisite for cell permissiveness to the infection. Retroviruses broaden their host range by mutations of the env gene, and vice versa, host cells develop resistance to retrovirus by mutations of genes encoding the specific receptors. We have described such an interesting semi-resistant phenotype in chicken line M and explained it by mutation of the receptor Tvb. Another defence mechanism used by the host cells is the inactivation of integrated invaders at the level of transcription via DNA methylation and modifications of adjacent histones. This was demonstrated in the case of HIV-1 latency, and inhibitors of DNA methyltransferases and histone deacetylases must be considered for HIV-1 eradication from the reservoir of long-lived, latently infected resting memory CD4$^+$ T cells. Another example of epigenetic regulation is represented by endogenous retroviruses in the human genome. Fusogenic envelope glycoproteins encoded by two copies of HERVs are strictly placenta-specific and their expression in other tissues must be prevented by DNA methylation and histone methylation. Epigenetic silencing is, however, an obstacle in using retroviruses as vectors for gene transfer and transgenesis. We have improved ASLV-based retroviral vectors by insertion of core element from CpG island between promoter and enhancer, which increases their resistance to transcriptional silencing and ensures long-term expression of transduced genes. Finally, we have identified two genomic copies of porcine endogenous retroviruses as a potential risk factor in xenotransplantation of pig organs and tissues.

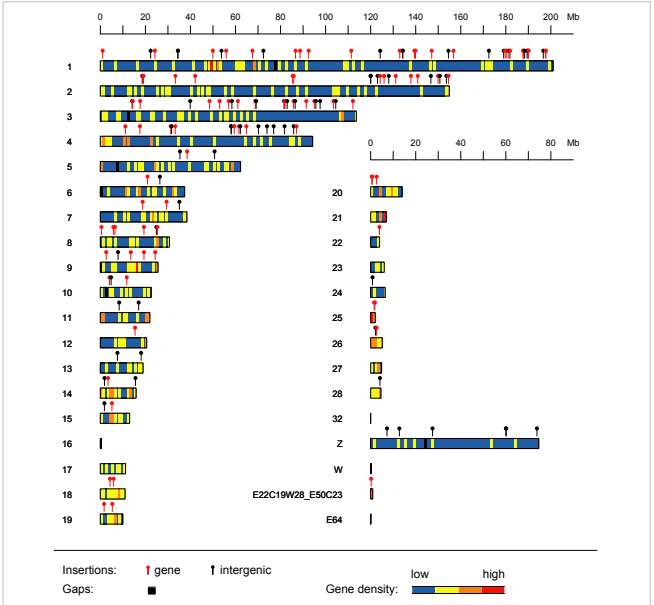

Fig. 1. Chromosomal locations of Rous sarcoma virus integrations in virus-induced chicken tumours. Integrations into and outside genes are shown as red and black lollipops, respectively.

- FP6 EU, 37377 XENOME – Engineering of the porcine genome for xenotransplantation studies in primates: a step towards clinical application, 2006-2011, J. Hejnar
- GA CR, GA523/07/1282 – Characterization of the cellular receptor Tvc and its role in the pathogenesis of avian leukosis viruses subgroup C-induced diseases, 2007-2009, J. Geryk
- GA CR, GA204/07/1030 – Integration preference of retroviruses, 2007-2009, J. Plachý
- GA CR, GA523/07/1171 – L1 retrotransposon-based transgenic chicken models, 2007-2009, J. Hejnar
- AS CR, IAA500520709 – Mechanisms of syncytin-mediated cell-to-cell fusion in placenta, 2007-2009, J. Hejnar
- GA CR, GP204/08/P616 – The role of DNA methylation and histone modifications in the establishment and maintenance of HIV-1 latency, 2008-2010, J. Blažková
- GA CR, GA301/09/2031 – Fusogenic envelope glycoproteins of human endogenous retroviruses and their receptors, 2009-2011, J. Hejnar
- GA CR, GP301/09/P667 – Transcriptional silencing of retroviral vectors during their integration into the host genome, 2009-2011, F. Šenigl
- GA CR, GAP502/10/1651 – Characterization of new avian sarcoma and leukosis virus (ASLV) receptor alleles as revealed by the analysis of semiresistant cells, 2010-2012, J. Plachý

1. Trejbalová K, Blažková J, Matoušková M, Pecnová L, Kučerová D, Vernerová Z, Heráček J, Hirsch I, Hejnar J. Epigenetic regulation of transcription and splicing of syncytins, fusogenic glycoproteins of retroviral origin (submitted).
2. Plachý J, Kotáb J, Divina P, Reinišová M, Šenigl F, Hejnar J. Proviruses selected for high and stable expression of transduced genes accumulate in broadly transcribed genome areas. **J Virol 2010** 84(9): 4204-4211.
3. Blazkova J, Trejbalova K, Gondois-Rey F, Halfon P, Philibert P, Guiguen A, Verdin E, Olive D, Van Lint C, Hejnar J, Hirsch I. CpG methylation controls reactivation of HIV from latency. **PLoS Pathog 2009** 5(8): e1000554.
4. Šenigl F, Plachý J, Hejnar J. The core element of a CpG island protects avian sarcoma and leukosis virus-derived vectors from transcriptional silencing. **J Virol 2008** 82(16): 7818-7827.
5. Reinišová M, Šenigl F, Yin X, Plachý J, Geryk J, Elleder D, Svoboda J, Federspiel MJ, Hejnar J. A single-amino-acid substitution in the TvbS1 receptor results in decreased susceptibility to infection by avian sarcoma and leukosis virus subgroups B and D and resistance to infection by subgroup E *in vitro* and *in vivo*. **J Virol 2008** 82(5): 2097-2105.

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Laboratory of Transplantation Immunology

Transplantation immunity, cytokines, stem cells, immunoregulation

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Transplantation of organ or transfer of stem cells often represents the only way to improve or even to rescue life. However, immunological rejection represents the major obstacle to further development of clinical transplantation. Therefore, deep knowledge of molecular and cellular mechanisms of the transplantation reaction is required. Using the model of immunological reaction to histocompatibility antigens we have focused on the study of activation and function of regulatory T cells in transplantation immunity and tolerance. On the model of orthotopic corneal and limbal transplantation we have analysed expression of genes for cytokines and other effector molecules during graft rejection and studied possibilities to prevent rejection of corneal and limbal grafts. Since successful treatment of damaged cornea requires transfer of limbal stem cells, we recently started to isolate, grow and characterize stem cells. We succeeded in isolating limbal and mesenchymal stem cells in the mouse and using them for the repair of damaged corneal epithelium. For the transfer of stem cells we use various types of nanofibre scaffolds, which represent optimal 3D matrices for stem cell growth. Well-established methods for monitoring the immune response enabled us, in co-operation with other laboratories, to study cytokine response in various experimental models of immunoregulation. The ultimate goal of our research is to get insights into the mechanisms of specific immune response, to isolate and transplant stem cells and to propose novel strategies for targeted immunoregulation.

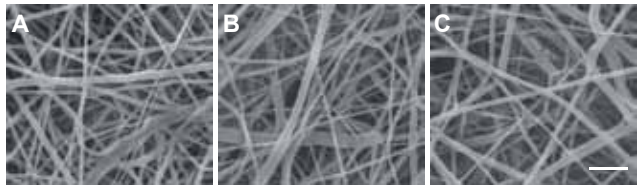

Fig. 1. Scanning electron microscopy of PA6/12 nanofibres used for culturing stem cells. Stability of the structure aqueous solution: before soaking (A) and preservation of the nanofibre architecture after one (B) or two (C) weeks soaking in aqueous solutions. Scale bar: 5 μm.

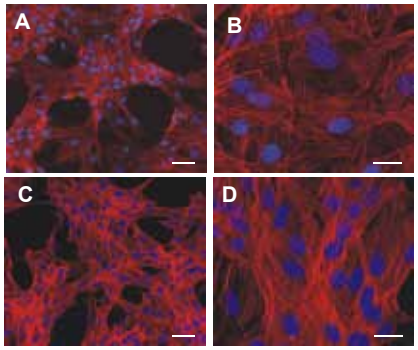

Fig. 2. The morphology of limbal stem cells (LSC) growing on a glass surface or on PA6/12 nanofibres. The cells were cultured for 24 h on poly L-lysine-coated glass inserts in 24-well tissue culture plates or on nanofibres fixed in the inserts and were stained for F-actin with phalloidin (red filaments). The nuclei are blue (DAPI staining). (A) and (B) demonstrate LSC growing on the glass surface at two different magnifications, (C) and (D) show LSC growing on nanofibre scaffolds. Scale bars: A, C – 50 μm, B, D – 20 μm.

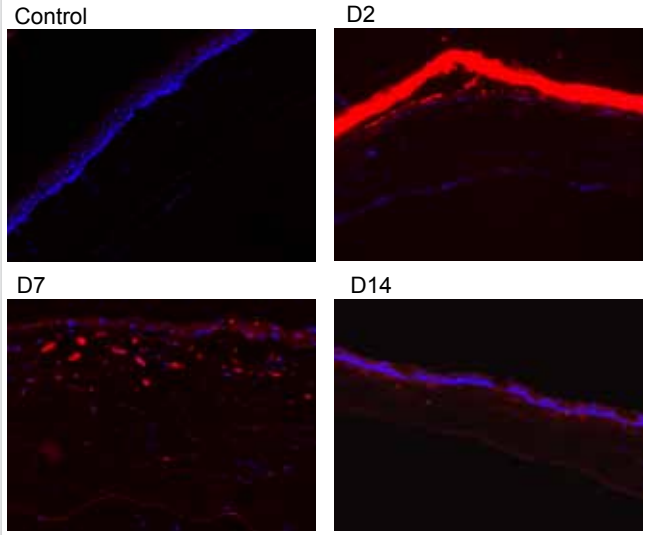

Fig. 3. Detection of PKH26-labelled limbal stem cells (LSC) on the damaged ocular surface after their transfer using nanofibre scaffold. The globes were removed 2, 7 or 14 days after the operation and 7 μm cryosections were prepared. The nuclei were visualized with DAPI. The cryosections were prepared from the control undamaged eye (without labelled cells), (D2) from the eye 2 days after operation (the nanofibre scaffold with labelled LSC is seen as a red lane, corneal epithelium is removed), and from the eyes 7 (D7) and 14 (D14) days after the cell transfer (red stained cells are still present, the corneal epithelium is regenerated). Magnification: 200x.

- FP7 EU, 222509 KINACEPT – Novel anti-inflammatory compounds for autoimmune diseases, 2008-2010, V. Holáň
- GA CR, GD310/08/H077 – Regulation of immunological mechanisms in health and disease: Development of new diagnostic and therapeutic approaches, 2008-2011, V. Holáň
- AS CR, KAN200520804 – Biocompatible nanofibre scaffolds forming novel drug matrices for the application of biologically and pharmacologically active substances, 2008-2012, V. Holáň
- GA CR, GA206/08/0640 – Immunogenetic study of the hybrid zone of the house mouse, 2008-2012, V. Holáň

1. Zajicova A, Pokorna K, Lencova A, Krulova M, Svobodova E, Kubinova S, Sykova E, Pradny M, Michalek J, Svobodova J, Munzarova M, Holan V. Treatment of ocular surface injuries by limbal and mesenchymal stem cells growing on nanofiber scaffolds. **Cell Transplant 2010** 19(10): 1281-1290.
2. Furugaki K, Pokorna K, Le Pogam C, Aoki M, Reboul M, Bajzik V, Krief P, Janin A, Noguera ME, West R, Charron D, Chomienne C, Pla M, Moins-Teisserenc H, Padua RA. DNA vaccination with all-trans retinoic acid treatment induces long-term survival and elicits specific immune responses requiring CD4+ and CD8+ T-cell activation in an acute promyelocytic leukemia mouse model. **Blood 2010** 115(3): 653-656.
3. Holan V, Pokorna K, Prochazkova J, Krulova M, Zajicova A. Immunoregulatory properties of mouse limbal stem cells. **J Immunol 2010** 184(4): 2124-2129.
4. Prochazkova J, Fric J, Pokorna K, Neuwirth A, Krulova M, Zajicova A, Holan V. Distinct regulatory roles of transforming growth factor-beta and interleukin-4 in the development and maintenance of natural and induced CD4+ CD25+ Foxp3+ regulatory T cells. **Immunology 2009** 128(1 Suppl): e670-678.
5. Lipoldová M, Havelková H, Badalová J, Vojtíšková J, Quan L, Krulová M, Sohrabi Y, Stassen AP, Demant P. Loci controlling lymphocyte production of interferon gamma after alloantigen stimulation *in vitro* and their co-localization with genes controlling lymphocyte infiltration of tumors and tumor susceptibility. **Cancer Immunol Immunother** 2009 Aug 5 [Epub ahead of print].

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Laboratory of Molecular Immunology

Transmembrane adaptor proteins, membrane rafts, leukocyte signalling proteins

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In recent years a major topic of our laboratory has been signalling molecules present in membrane rafts, namely several transmembrane adaptor proteins discovered previously by us (PAG/Cbp, NTAL/LAB, LIME) and their involvement in immunoreceptor signalling. In 2009-2010 we worked on elucidation of the structure and function of an apparently novel type of "heavy" rafts, differing from the "classical" ones by higher protein-lipid ratio and containing a number of transmembrane proteins. We continued our studies on several novel raft-associated transmembrane adaptors (LST1A, PRR7, Nvl), targeting of protein tyrosine kinase Csk into various membrane compartments, on receptor phosphatase CD148, and collaborated on several studies concerning membrane rafts and their components. Furthermore, we produced a number of novel monoclonal antibodies as valuable research and potentially diagnostic tools, e.g. those to LARGE, CLIC 5, OPAL 1, DDIT4L.

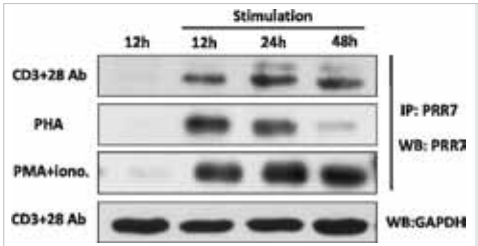

Fig. 1. Kinetics of PRR7 protein expression in human T lymphocytes activated by different means.

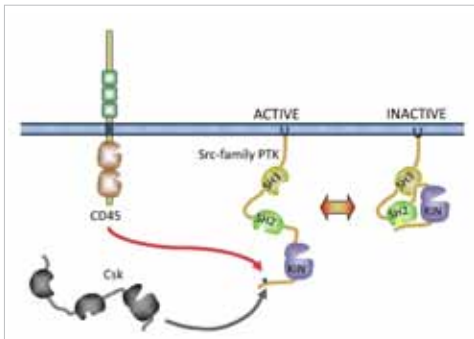

Fig. 2. Schematic representation of Src family kinase regulation by membrane phosphatase CD45 and tyrosine kinase Csk.

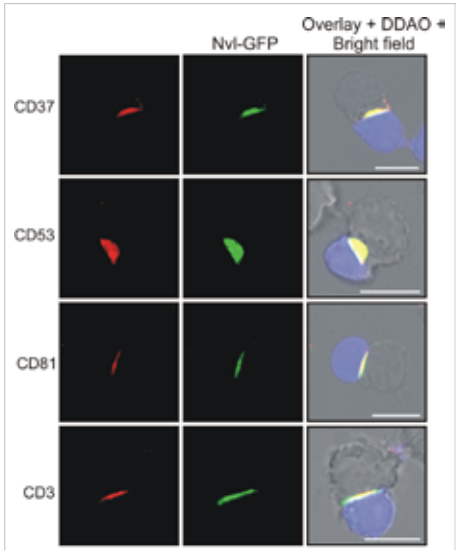

Fig. 3. Co-localization of transmembrane adaptor protein Nvl, tetraspanin proteins CD37, CD53, CD81 and CD3 in immunological synapse formed between antigen-presenting cell and T cell.

- Ministry of Education, Youth and Sports of the Czech Republic, 1M0506 – Centre of Molecular and Cellular Immunology, 2005-2011, V. Hořejší
- Ministry of Education, Youth and Sports of the Czech Republic, 2B06064 – New target genes for childhood leukaemia diagnosis and treatment focused on adaptor molecules of signalling pathways, 2006-2011, T. Brdička
- GA CR, GEMEM/09/E011 – Signalling and adaptor proteins of leukocyte membrane microdomains, 2009-2012, V. Hořejší

1. Štěpánek O, Kalina T, Dráber P, Skopcová T, Švojgr K, Angelisová P, Hořejší V, Weiss A, Brdička T. Regulation of Src-family kinases involved in T-cell receptor signaling by protein tyrosine phosphatase CD148 (in revision).
2. Ormsby T, Schlecker E, Ferdin J, Tessarz AS, Angelisová P, Köprülü AD, Borte M, Warnatz K, Ehl S, Ellmeier W, Hořejší V, Cerwenka A. Btk is a positive regulator in the TREM-1/DAP12 signaling pathway (in revision).
3. Otáhal P, Pata S, Angelisová P, Hořejší V, Brdička T. The effects of membrane compartmentalization of Csk kinase on TCR signaling. **Biochim Biophys Acta 2011** 1813(2): 367-376.
4. Hořejší V, Otáhal P, Brdička T. LAT – an important raft-associated transmembrane adaptor protein. **FEBS J 2010** 227(21): 4383-4397 (invited review).
5. Otahál P, Angelisová P, Hrdinka M, Brdička T, Novák P, Drbal K, Hořejší V. A new type of membrane raft-like microdomains and their possible involvement in TCR signaling. **J Immunol 2010** 184(7): 3689-3696.

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Laboratory of Biology of the Cell Nucleus

Cell nucleus, nucleoskeleton, nuclear actin, myosin, microscopy, ultrastructural methods

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In diploid mammalian cells, some $6x10^9$ base pairs of DNA fold as a nucleoprotein complex (i.e. chromatin) into higher-order arrays so as to fit in a nucleus measuring only 10 μm. The nucleus also contains machineries for transcription of genes and processing of RNA products, and for precise DNA replication, repair and recombination. Nuclear interior is therefore functionally highly compartmentalized, and the recent evidence points strongly to structure-related regulation of nuclear functions – however, the mechanisms forming the 3D-structure of the nucleus are still mostly obscure. We therefore employ a multi-disciplinary approach in order to study nuclear functions in relation to the higher-order nuclear structures, e.g. nuclear bodies, the nucleolus, and the nucleoskeleton. Our research concentrates on: (1) the relationship between nuclear compartmentalization and regulation of gene expression, (2) structure, dynamics, and function of the nucleoskeleton, which might direct nuclear compartmentalization, (3) functions of nuclear myosin I and actin in transcription and gene expression, (4) development of new microscopy methods for ultrastructural studies.

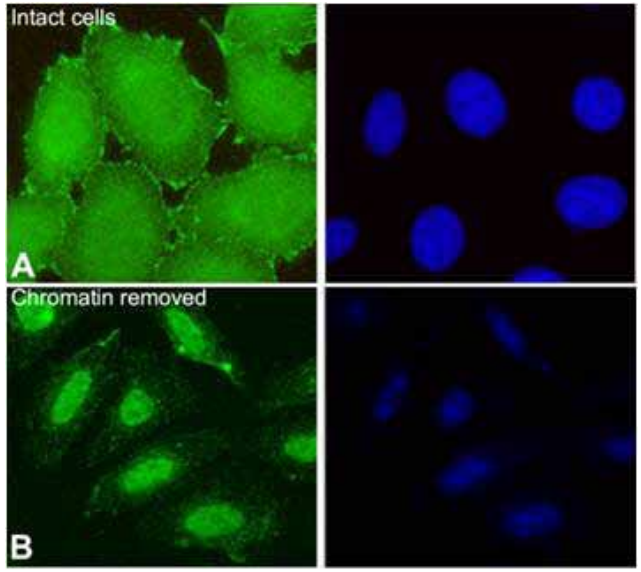

Fig. 1. Vinculin is an actin-binding protein which is considered to be exclusively cytoplasmic. We demonstrate for the first time nuclear localization of vinculin in intact cells (A). The intensity of the signal becomes stronger after the removal of chromatin (B). Green: anti-vinculin antibody; blue: DNA.

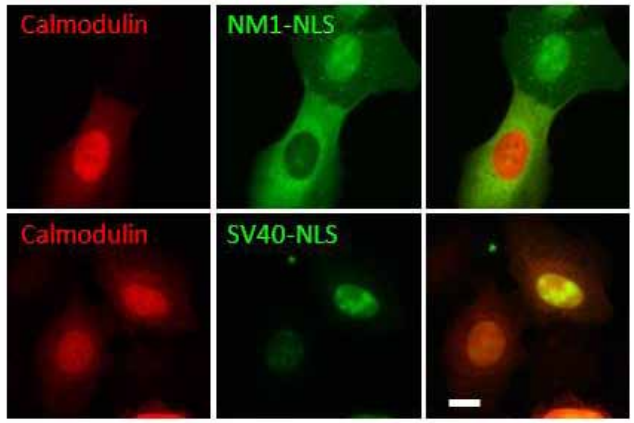

Fig. 2. Nuclear transport mediated by NM1 nuclear localizing signal (NLS) is inhibited by calmodulin. This suggests that NM1 transport to the nucleus might be regulated by calcium levels.

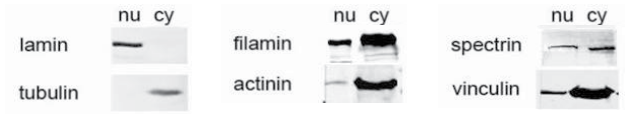

Fig. 3. Various actin-binding proteins were identified in nuclear extracts of Hela cells. Lamin and tubulin antibodies were used for control of purity of nuclear (nu) and cytoplasmic (cy) fractions. This information forms a basis for studying new components of nuclear structures.

- Ministry of Education, Youth and Sports of the Czech Republic, LC545 – Functional Organisation of the Cell, 2005-2011, P. Hozák
- Ministry of Education, Youth and Sports of the Czech Republic, LC06063 – Fluorescence Microscopy in Biological and Medical Research, 2006-2011, P. Hozák
- Ministry of Education, Youth and Sports of the Czech Republic, 2B06063 – Structure and irregularities of the nucleoskeleton: identification and diagnostic, 2006-2011, P. Hozák
- AS CR, KAN200520704 – New nanoparticles for ultrastructural diagnostics, 2007-2011, P. Hozák
- Ministry of Education, Youth and Sports of the Czech Republic, ME09101 – Cooperative contribution of actin- and myosin-families to the chromatin dynamics and transcription in the cell nucleus, 2009-2010, P. Hozák
- GA CR, GD204/09/H084 – Molecular biology of a differentiating cell, 2009-2012, P. Hozák

1. Philimonenko VV, Janáček J, Harata M, Hozák P. Transcription-dependent rearrangements of actin and nuclear myosin I in the nucleolus. **Histochem Cell Biol 2010**, 134, 243-249.
2. Castano E, Philimonenko VV, Kahle M, Fukalová J, Kalendová A, Yildirim S, Dzijak R, Dingová-Krásna H, Hozák P. Actin complexes in the cell nucleus: new stones in an old field. **Histochem Cell Biol 2010**, 133, 607-626.
3. Novakova Z, Hubackova S, Kosar M, Janderova-Rossmeislova L, Dobrovolna J, Vasicova P, Vancurova M, Horejsi Z, Hozak P, Bartek J, Hodny Z. Cytokine expression and signaling in drug-induced cellular senescence. **Oncogene 2010** 29(2): 273-84.
4. Dingová H, Fukalová J, Maninová M, Philimonenko VV, Hozák P. Ultrastructural localization of actin and actin-binding proteins in the nucleus. **Histochem Cell Biol 2009** 131(3): 425-434.
5. Fulka H, St John JC, Fulka J, Hozák P. Chromatin in early mammalian embryos: achieving the pluripotent state. **Differentiation 2008** 76(1): 3-14.

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Laboratory of Chromosomal Stability

DNA damage response, DNA repair, genomic instability, cancer

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DNA damage is a frequent event in the life of a cell. Failure to repair DNA damage can lead to cell death, while inaccurate DNA repair can give rise to genomic instability, which promotes the onset of cancer in mammals. Proteins belonging to the RecQ family of DNA helicases play important roles in the maintenance of genomic stability in all kingdoms of life, which is highlighted by the finding of an association of inherited defects in three human RecQ helicases, namely BLM, WRN and RECQ4, with distinct autosomal recessive disorders characterized by genomic instability and cancer predisposition. The main goal of the research in our laboratory is to provide clear understanding of the functions of these enzymes in mammalian cells. Our current studies focus on RECQ4, which is mutated in Rothmund-Thompson syndrome, a severe disorder manifested by photosensitivity, skeletal abnormalities, aneuploidy, chromosomal rearrangements and predisposition to osteosarcomas. A number of recent studies have demonstrated that RECQ4 is essential for the initiation of DNA replication. However, RECQ4 also accumulates at DNA double-strand breaks (DSBs) and interacts with the RAD51 recombinase that mediates homology search and strand exchange during DSB repair by homologous recombination (HR). Our aim is to explore the role of RECQ4 in DNA DSB repair.

Although HR between sister chromatids provides the most accurate mechanism for repair of DSBs, it has to be tightly regulated, especially at the strand invasion step, to prevent recombination events between homologous sequences at different chromosomal loci, which can give rise to chromosomal rearrangements. A number of DNA helicases including FBH1 have been implicated in the regulation of HR, but the underlying mechanisms remain elusive. Another goal of the laboratory is to elucidate the mechanistic basis of the anti-recombinase function of FBH1.

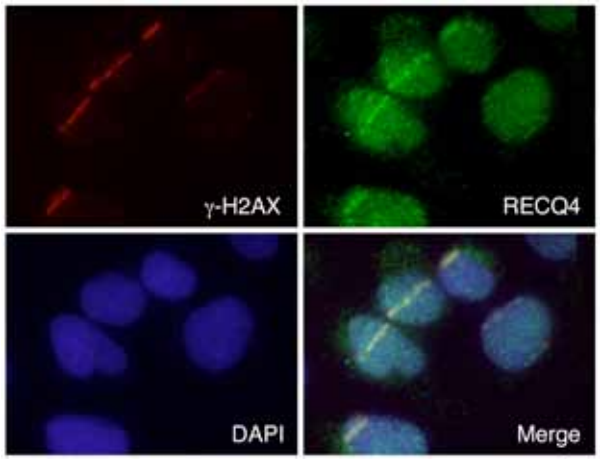

Fig. 1. Accumulation of the human RECQ4 protein at DNA double-strand breaks generated by laser-microirradiation.

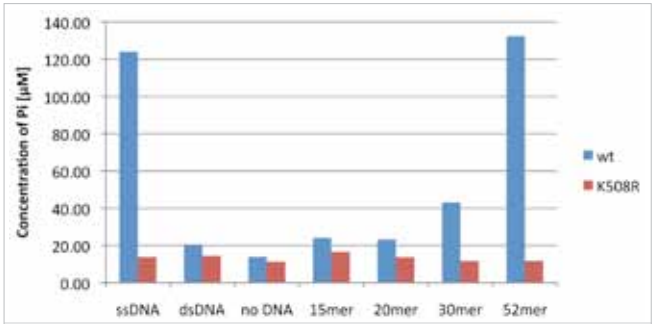

Fig. 2. ATPase activity of RECQ4 in the presence of different DNA cofactors.

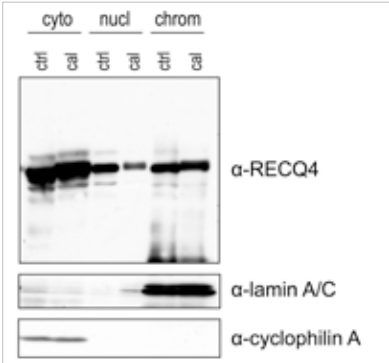

Fig. 3. Western blot analysis of cytoplasmic, nucleoplasmic and chromatin fractions of human U2OS cells treated or not for 20 minutes with 100 mM calyculin A (cal), a serine/threonine phosphatase inhibitor.

- GA CR, GA204/09/0565 – Role of RECQ5 DNA helicase in maintenance of genomic stability, 2009-2013, P. Janščák
- GA CR, GAP305/10/0281 – Role of the Rothmund-Thomson syndrome gene product in maintenance of genomic stability, 2010-2014, P. Janščák

1. Kanagaraj R, Huehn D, Mackellar A, Menigatti M, Zheng L, Urban V, Shevelev I, Greenleaf AL, Janscak P. RECQ5 helicase associates with the C-terminal repeat domain of RNA polymerase II during productive elongation phase of transcription. **Nucleic Acids Res 2010** 38(22): 8131-8140.
2. Schwendener S, Raynard S, Paliwal S, Cheng A, Kanagaraj R, Shevelev I, Stark JM, Sung P, Janscak P. Physical interaction of RECQ5 helicase with RAD51 facilitates its anti-recombinase activity. **J Biol Chem 2010** 285(21): 15739-15745.
3. Dietschy T, Shevelev I, Pena-Diaz J, Huhn D, Kuenzle S, Mak R, Miah MF, Hess D, Fey M, Hottiger MO, Janscak P, Stagljar I. p300-mediated acetylation of the Rothmund-Thomson-syndrome gene product RECQL4 regulates its subcellular localization. **J Cell Sci 2009** 122(Pt 8): 1258-1267.
4. Zheng L, Kanagaraj R, Mihaljevic B, Schwendener S, Sartori AA, Gerrits B, Shevelev I, Janscak P. MRE11 complex links RECQ5 helicase to sites of DNA damage. **Nucleic Acids Res 2009** 37(8): 2645-2657.
5. Yodh JG, Stevens BC, Kanagaraj R, Janscak P, Ha T. BLM helicase measures DNA unwound before switching strands and hRPA promotes unwinding reinitiation. **EMBO J 2009** 28(4): 405-416.

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Laboratory of Cell and Developmental Biology

Colorectal cancer, Wnt signalling, TCF/LEF transcription factors, Hypermethylated in Cancer 1, HIC1

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The majority of tissues in the adult organism contain a population of tissue-specific stem cells. These multipotent cells are involved in homeostatic self-renewal and tissue repair processes. The biology of the stem cells is driven by a limited set of signalling cascades. The deregulation of these cascades can ultimately lead to the cellular transformation and formation of tumours. This clearly indicates the connection between the stem cell physiology and cancer. The scientific goal of the laboratory is to elucidate molecular mechanisms influencing behaviour of normal and diseased intestinal epithelial cells. Since the fate of these cells is determined by the so-called Wnt signalling pathway, our main focus is to find genes regulated by the Wnt pathway and/or encoding proteins directly involved in the signalling process. The important result in the current years was the identification of the HIC1 (Hypermethylated In Cancer 1) tumour suppressor as a novel modulator of the Wnt signalling cascade. Moreover, using various molecular biology approaches we discovered several other proteins (e.g. Dazap2 and Troy) that participate in Wnt signalling or act in downstream molecular events triggered by active Wnt signalling. Currently, the laboratory used the gene targeting technology in mouse embryonic stem cells to produce a novel mouse strain containing a so-called conditional allele of the Hic1 gene. Furthermore, we generated several "reporter" mice allowing lineage tracing experiments in mouse embryonic and adult tissues.

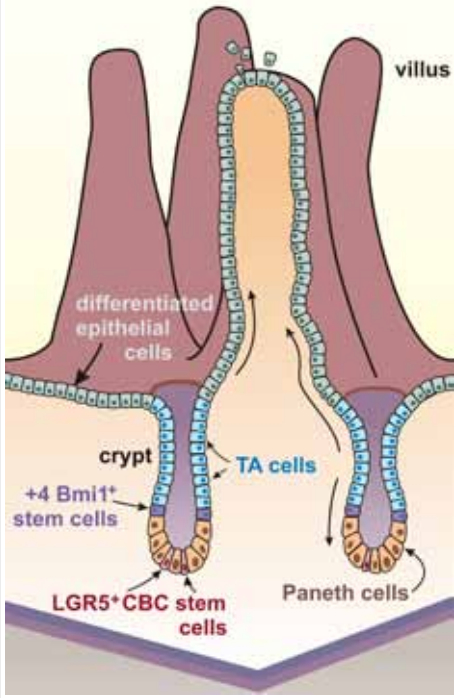

Fig. 1. Epithelium of adult small intestine. TA, rapidly dividing transit-amplifying cells (adopted from Reya and Clever, Nature, 2005).

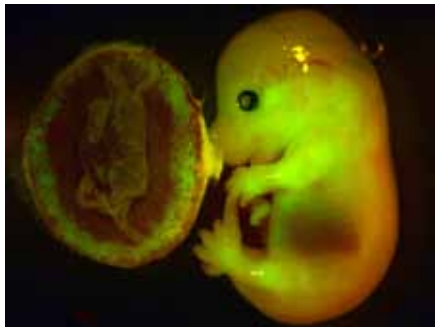

Fig. 2. Hic1 reporter mouse. The picture shows a mouse embryo at E 14.5 and its placenta. The animal was generated by a "knockin" of the enhanced yellow fluorescent protein (EYFP) into the Hic1 locus. These mice allow tracing Hic1 expression using EYFP-derived fluorescence as the surrogate marker. Notice the positive signal in the skeletal elements of the embryo and the junctional zone of the placenta.

Fig. 3. The lineage tracing experiment in the small intestine. The section of the gut tissue derived from the Troy-CreERT2 transgenic mice crossed with Rosa26-STOP-lacZ reporter mice 24 hour after tamoxifen injection. The blue stainings corresponding to the expression of the Troy gene is mainly found in the putative stem cells residing at the bottom of the crypts.

- Ministry of Education, Youth and Sports of the Czech Republic, 2B06077 – High throughput analysis of chromatin structure for development of novel diagnostic and therapeutic approaches in cancer, 2006-2011, V. Kořínek
- Ministry of Education, Youth and Sports of the Czech Republic, 1M0506 – Centre for Molecular and Cellular Immunology, 2005-2011, V. Kořínek
- GA CR, GA204/07/1567 – Tumour suppressors in the Wnt signalling pathway, 2007-2010, V. Kořínek
- GA CR, GD204/09/H058 – Intercellular signalling in development and disease; 2009-2012, V. Kořínek, J. Turečková
- GA Charles University, 20210 – Interaction of HIC1 and APC, two tumour suppressors involved in Wnt signalling, 2010-2011, V. Pospíchalová

1. Turečková J, Vojtěchová M, Krausová M, Šloncová E, Korínek V. Focal adhesion kinase functions as an akt downstream target in migration of colorectal cancer cells. **Transl Oncol 2009** 2(4): 281-290.
2. Klíma M, Zájedová J, Doubravská L, Anděra L. Functional analysis of the posttranslational modifications of the death receptor 6. **Biochim Biophys Acta 2009** 1793(10): 1579-1587.
3. Fujimura N, Taketo MM, Mori M, Korinek V, Kozmik Z. Spatial and temporal regulation of Wnt/beta-catenin signaling is essential for development of the retinal pigment epithelium. **Dev Biol 2009** 334(1): 31-45.
4. Lukas J, Mazna P, Valenta T, Doubravska L, Pospichalova V, Vojtechova M, Fafilek B, Ivanek R, Plachy J, Novak J, Korinek V. Dazap2 modulates transcription driven by the Wnt effector TCF-4. **Nucleic Acids Res 2009** 37(9): 3007-3020.
5. Doubravská L, Šímová Š, Cermak L, Valenta T, Kořínek V, Anděra L. Wnt-expressing rat embryonic fibroblasts suppress Apo2L/TRAIL-induced apoptosis of human leukemia cells. **Apoptosis 2008** 13(4): 573-587.

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Laboratory of Transcriptional Regulation

Eye development and evolution, Pax genes, Wnt/β-catenin signalling

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We are interested in the genetic basis of mammalian eye development. Our focus is on the role of transcription factors and signalling cascades, especially on the role of *Pax6* gene, Wnt/β-catenin signalling pathway and their genetic interaction. A combination of gain-of-function (transgenic) and loss-of-function (conditional knock-outs) approaches is used. Our second main interest is eye evolution. Early morphological studies have suggested that eye has evolved multiple times during the course of evolution. In contrast, more recent genetic data indicate a conserved role of *Pax6* and some other transcription factors in eye formation in a wide range of animals. In fact, eye assembly always relies on the same basic principle, i.e. photoreceptors located in the vicinity of dark shielding pigment. Several model systems including amphioxus, scallop, medaka and jellyfish are used in the laboratory to study various aspects of eye evolution.

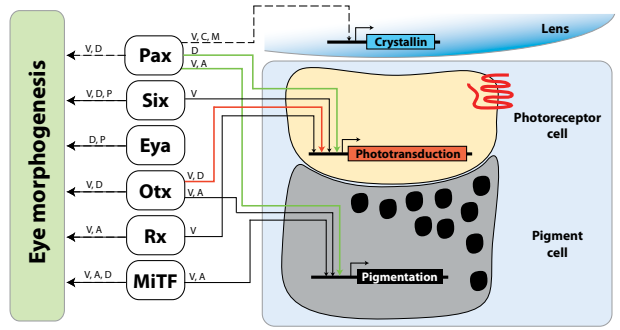

Fig. 1. Dual role of transcription factors in regulation of both eye development and differentiation genes (Vopalensky and Kozmik, 2009).

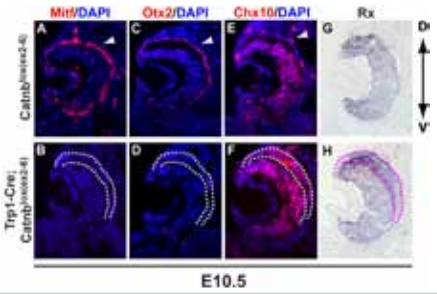

Fig. 2. Elimination of Wnt/β-catenin signalling in the developing retinal pigment epithelium (RPE) leads to tissue hyperproliferation and transdifferentiation of RPE into neural retina. Please note the loss of RPE markers (Otx2, Mitf) and the gain of neural retina-specific markers (Chx10, Rx) (Fujimura *et al.*, 2009).

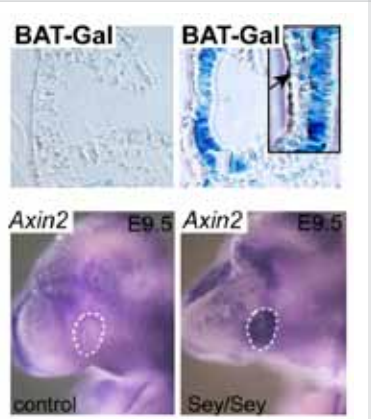

Fig. 3. Wnt/β-catenin signalling is ectopically activated in Pax6-deficient (Sey/Sey) mouse embryos as exemplified by the upregulation of Wnt-sensitive reporter gene *BAT-gal* and Wnt target gene *Axin2* (Machon *et al.*, 2010).

- AS CR, IAA500520604 – Developmental genetics of amphioxus: insight into evolutionary origin of vertebrates, 2006-2010, Z. Kozmik
- GA CR, GA204/08/1618 – Molecular basis of canonical Wnt signalling during eye and brain development, 2008-2010, Z. Kozmik
- GA CR, GD204/09/H058 – Intercellular signalling in development of the organism and disease, 2009-2012, Z. Kozmik
- AS CR, IAA500520908 – The role of Pax genes in eye evolution, 2009-2013, Z. Kozmik
- GA CR, GCP305/10/J064 – Reconstructing urbilaterian photoreceptors: comparative study between Branchiostoma (Chordata) and Platynereis (Annelida), 2010-2013, Z. Kozmik

1. Machon O, Kreslova J, Ruzickova J, Vacik T, Klimova L, Fujimura N, Lachova J, Kozmik Z. Lens morphogenesis is dependent on Pax6-mediated inhibition of the canonical Wnt/β-catenin signaling in the lens surface ectoderm. **Genesis 2010** 48(2): 86-95.
2. Ruzickova J, Piatigorsky J, Kozmik Z. Eye-specific expression of an ancestral jellyfish PaxB gene interferes with Pax6 function despite its conserved Pax6/Pax2 characteristics. **Int J Dev Biol 2009** 53(4): 469-482.
3. Fujimura N, Taketo MM, Mori M, Korinek V, Kozmik Z. Spatial and temporal regulation of Wnt/-catenin signaling is essential for development of the retinal pigment epithelium. **Dev Biol 2009** 334(1): 31-45.
4. Vopalensky P, Kozmik Z. Eye evolution: common use and independent recruitment of genetic components. **Phil Trans R Soc Lond B Biol Sci 2009** 364(1531): 2819-2832.
5. Kozmik Z, Ruzickova J, Jonasova K, Matsumoto Y, Vopalensky P, Kozmikova I, Strnad H, Kawamura S, Piatigorsky J, Paces V, Vlcek C. Assembly of the cnidarian camera-type eye from vertebrate-like components. **Proc Natl Acad Sci USA 2008** 105(26): 8989-8993.

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Laboratory of Molecular and Cellular Immunology

Functional gene mapping, leishmaniasis, atopy

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The research programme of the laboratory aims to identify genes and molecular mechanisms involved in control of immune response and susceptibility to complex infectious diseases. We focus on complex diseases because they are responsible for the largest part of human morbidity and mortality and their genetic analysis is subject of an intensive international effort. They are controlled by multiple genes and hence their pathogenesis cannot be explained by effects of a single gene with omission of others. Leishmaniasis is such a complex disease and it has served as a major paradigm of immune response to an infectious agent. We aim to identify the genes and functions controlling this disease. The disease is caused by protozoan parasites of genus *Leishmania* that multiply in macrophages. Different species of *Leishmania* induce different symptoms, but even the patients infected by the same species develop different clinical manifestations. Many phenomena observed in human leishmaniases can be investigated in *Leishmania major* infection in mouse. Our approach uses a combination of genetic dissection with screening of a large set of immunological and clinical parameters of the disease. We mapped 21 *Lmr* (*Leishmania major* response) loci and found that gene effects on disease symptoms were organ-specific and heterogeneous. These 21 individual *Lmr* loci control 17 different combinations of pathological and immunological symptoms. Eight loci control both organ pathology and immunological parameters and 13 only immune reactions. Fifteen *Lmr* loci are involved in one or more genetic interactions showing that gene interactions are common in response to *L. major*. Moreover, parasite elimination, immunological and pathological processes are regulated independently. In conclusion, these studies revealed a network-like complexity of the combined effects of the multiple functionally diverse QTLs (quantitative trait loci).

Lmr loci are likely relevant also for other diseases. Interestingly, nine of ten *Lmr* that influence serum IgE level after *Leishmania major* infection were mapped in the regions homologous with the human chromosomal segments that control total serum IgE in human atopic diseases. However, for the Lmr9 locus, the homologous human regions have not been connected with atopy. Thus, this locus may point to hitherto undetected human genes that are relevant for atopy. Indeed, in the position homologous to *Lmr9* on chromosome 8q12 we demonstrated a novel human IgE-controlling locus. This finding shows precision and predictive power of mouse models in investigation of complex traits in humans.

Fig. 1. Cutaneous leishmaniasis.

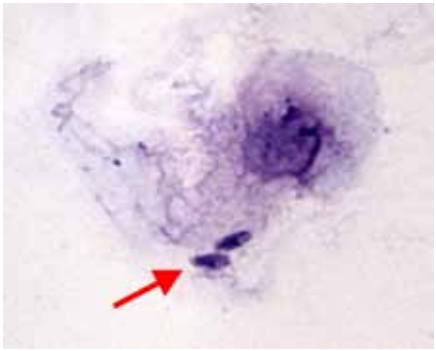

Fig. 2. *Leishmania* parasites in macrophage.

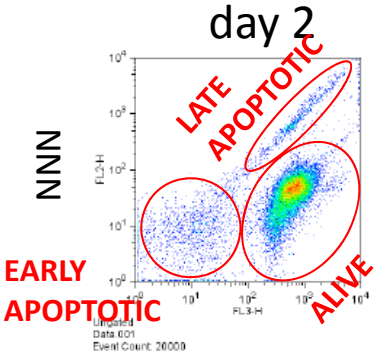

Fig. 3. *Leishmania* stained with propidium iodide and LDS 751.

- Ministry of Education, Youth and Sports of the Czech Republic, LC06009 – Centre for Molecular Ecology of Vectors and Pathogens, 2006-2011, M. Lipoldová
- GA CR, GA310/08/1697 – First genetic model for analysis of susceptibility to parasite Leishmania tropica: potential implications for studies of human leishmaniases, 2008-2012, M. Lipoldová

1. Kobets T, Badalová J, Grekov I, Havelková H, Svobodová M, Lipoldová M. *Leishmania* parasite detection and quantification using PCR-ELISA. **Nat Protoc 2010** 5(6):1074-1080.
2. Lipoldová M, Havelková H, Badalová J, Vojtíšková J, Quan L, Krulová M, Sohrabi Y, Stassen AP, Démant P. Loci controlling lymphocyte production of interferon gamma after alloantigen stimulation *in vitro* and their co-localization with genes controlling lymphocyte infiltration of tumors and tumor susceptibility. **Cancer Immunol Immunother 2010** 59 (2): 203-213.
3. Gusareva ES, Havelková H, Blažková H, Kosařová M, Kučera P, Král V, Salyakina D, Muller-Myhsok B, Lipoldová M. Mouse to human comparative genetics reveals a novel immunoglobulin E-controlling locus on Hsa8q12. **Immunogenetics 2009** 61(1): 15-25.
4. Kurey I, Kobets T, Havelková H, Slapničková M, Quan L, Trtková K, Grekov I, Svobodová M, Stassen AP, Hutson A, Démant P, Lipoldová M. Distinct genetic control of parasite elimination, dissemination, and disease after *Leishmania major* infection. **Immunogenetics 2009** 61(9): 619-633.
5. Gusareva ES, Ogorodova LM, Chernyak BA, Lipoldová M. Relationship between total and specific IgE in patients with asthma from Siberia. **J Allergy Clin Immunol 2008** 121(3): 781.

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Marie Lipoldová, Assoc Prof, PhD / Head of Laboratory

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Elena Gusareva, PhD / Postdoc
Gizela Koubková, PhD / Research Fellow [maternity leave]
Iva Rohoušová, PhD / Research Fellow [part time]
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As a long-term research programme of our laboratory we have been investigating the mechanisms by which tumour cells are capable to escape from immune responses. Our projects are focused on regulation of genes encoding molecules crucial for antigen presentation and for tumour cell elimination by specific immune responses, on mechanisms of immunosuppression and its possible overcoming, and, finally, on experimental anti-tumour immunotherapy. Most of our studies employ murine models for tumours associated with human papilloma virus (aetiological agent of the cervical carcinoma). Special attention has been paid to the setting of the minimal residual tumour disease treatment after primary tumour resection or chemotherapy.

MHC class I deficiency on tumour cells is a frequent mechanisms by which tumour cells can escape from specific immune responses. We have been interested in mechanisms underlying reversible MHC class I downregulation on tumour cells. We have found that epigenetic agents induce expression of genes involved in antigen-processing machinery and surface expression of MHC class I molecules on tumour cells, as well as of selected co-stimulatory and co-inhibitory molecules. Further analysis revealed that MHC class I downregulation in our model cell lines was associated with epigenetic silencing of antigen-presenting machinery genes. Our current projects in this field are focused on *in vivo* experiments with the aim to optimize immunotherapy of MHC class I-deficient tumours combined with administration of DNA methyltransferase inhibitors. We are also interested in epigenetic mechanisms underlying regulation of genes encoding antigen-presentation machinery genes, as well as co-stimulatory/inhibitory genes in antigen-presenting or regulatory immunocytes.

Our next areas of interest are populations of immunoregulatory cells (NKT, T-regulatory and myeloid-derived suppressor cells) and their dynamics in the course of tumour growth and therapy, as well as their mutual interactions. Recently, we have shown that administration of a glycolipid ligand activating NKT cells, β-galactosylceramide, inhibited growth of recurrent MHC class I-positive and -deficient tumours after surgery.

Along with these projects we have been interested in experimental anti-tumour immunotherapy and vaccines. We have been investigating the impacts of several chemotherapeutic agents on anti-tumour immunity and on regulatory cell populations. We have used cell and gene therapy approaches and dendritic cell-based vaccines, as well as genetically modified tumour cells producing cytokines (especially IL-12-producing cells) for vaccination and immunotherapy optimization.

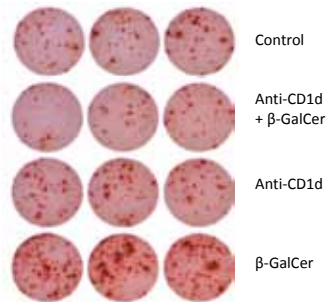


Fig. 1. ELISPOT (Enzyme-linked immunosorbent spot) analysis of the spleen cell activation with a ligand for NKT T-cell receptor (C12 β-D-GalactosylCeramide) and its inhibition with the anti-CD1d-antibody demonstrates that the C12 β-D-GalactosylCeramide effects are mediated through its presentation by the CD1d molecules. Each colour spot represents one interferon γ-producing spleen cell.

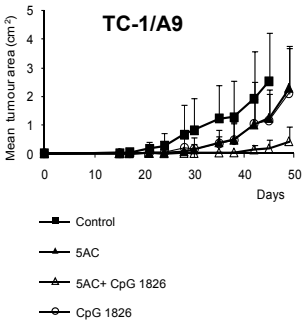
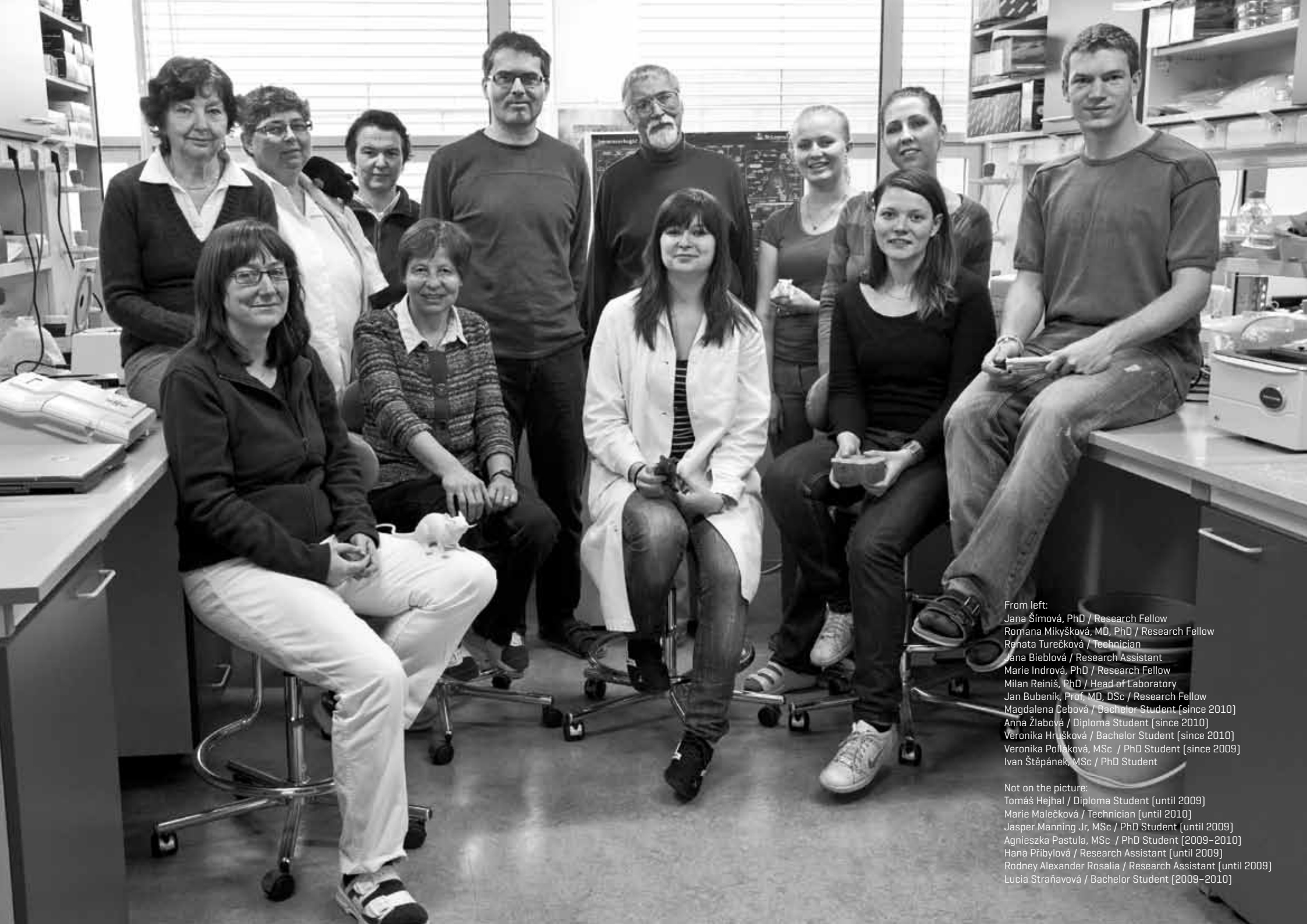


Fig. 2. Tumour growth curves in control mice and mice subjected to chemo- and immunotherapy and their combination. Tumour inhibitory effects of combined epigenetic agents, 5-azacytidine (5AC) and immune activator unmethylated oligodeoxynucleotide (CpG 1826). MHC class I-deficient tumour cells were transplanted on day 0. In experimental groups, 5AC was repeatedly administered on days 3, 7, 10, 14, 17, 21, 24, 28; immune activator, CpG 1826 was administered on days 3 and 10. Significant inhibition was observed in all treated mice, as compared to the untreated controls. Combined therapy was significantly more effective as compared to monotherapies only.

- FP6 EU, 18933 CLINIGENE – European network for the advancement of clinical gene transfer and therapy, 2006-2011, J. Bubeník
- AS CR, IAA500520605 – Induction of immunity against HPV16 E6/E7 oncogenes-induced tumours with peptide- and dendritic cell-based vaccines; immunotherapy of minimal residual tumour disease, 2006-2009, M. Reiniš
- GA CR, GA301/07/1410 – Immunosuppressive cell populations in the course of tumour progression and therapy, 2007-2011, M. Reiniš
- AS CR, IAA500520807 – Mechanisms of protective immunity against tumours with different expression of immunodulatory molecules, 2008-2010, M. Indrová
- Ministry of Health of the Czech Republic, NS10660 – Development of experimental anti-WT 1 vaccine for immunotherapy of tumours, 2009-2011, M. Indrová
- GA CR, GA301/09/1024 – Molecular and cellular mechanisms of tumour chemotherapy: immunomodulatory effect, 2009-2011, M. Indrová
- GA CR, GAP301/10/2174 – Epigenetic mechanisms in regulation of genes important for antigen presentation and antitumour immunity, 2010-2013, M. Reiniš

1. Reinis M, Stepanek I, Simova J, Bieblova J, Pribylova H, Indrova M, Bubenik J. Induction of protective immunity against MHC class I-deficient, HPV16-associated tumours with peptide and dendritic cell-based vaccines. **Int J Oncol 2010** 36(3): 545-551.
2. Šímová J, Indrová M, Bieblová J, Mikyšková R, Bubeník J, Reiniš M. Therapy for minimal residual tumor disease: β-Galactosylceramide inhibits the growth of recurrent HPV16-associated neoplasms after surgery and chemotherapy. **Int J Cancer 2010** 126(12): 2997-3004.
3. Indrová M, Bieblová J, Rossowska J, Kuropka P, Pajtasz-Piasecka E, Bubeník J, Reinis M. HPV 16-associated tumours: IL-12 can repair the absence of cytotoxic and proliferative responses of tumour infiltrating cells after chemotherapy. **Int J Oncol 2009** 34(1): 173-179.
4. Bubenik J. Genetically modified cellular vaccines for therapy of human papilloma virus type 16 (HPV 16)-associated tumours. **Curr Cancer Drug Targets 2008** 8(3): 180-186.
5. Manning J, Indrová M, Lubyová B, Přibylová H, Bieblová J, Hejnar J, Šímová J, Jandlová T, Bubeník J, Reiniš M. Induction of MHC class I molecule cell surface expression and epigenetic activation of antigen-processing machinery components in a murine model for human papilloma virus 16-associated tumours. **Immunology 2008** 123(2): 218-227.



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Laboratory of Structural Biology

Protein crystallography, HIV protease, antibody engineering

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Our laboratory carries out structural studies on various proteins of biological or medicinal interest using protein crystallography. Among our targets, proteases from pathogenic organisms [2-4], antibody fragments as well as other human enzymes [1] take a prominent position.

HIV protease [HIV PR] research is focused on development of novel potent inhibitors as well as on understanding the structural basis of drug resistance. More than two decades into the global HIV epidemic, HIV PR still remains an attractive target for structure-based rational drug design. Although nine inhibitors targeting HIV PR are currently approved for clinical use, their therapeutic efficiency is hampered mostly by resistance development. Understanding PR resistance at the structural level and development of new PIs acting by an alternative mode of inhibition is thus essential for successful treatment of HIV-positive patients. Our recent contribution to the field comprises structural studies of the enzyme-inhibitor complexes [2, 3].

Several recombinant antibody fragments of potential diagnostic and/or immunotherapeutic use [e.g. against human carbonic anhydrase IX, CD44, and CD3] have been prepared and characterized in our laboratory with the aim to improve their radionuclide labelling or to introduce further useful properties.

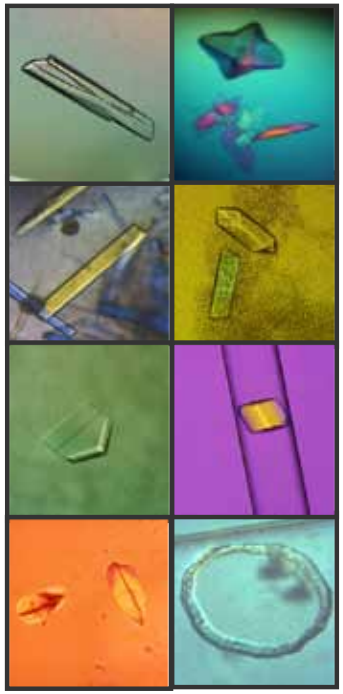

Fig. 1. Examples of protein crystals used for diffraction analysis and structure determination.

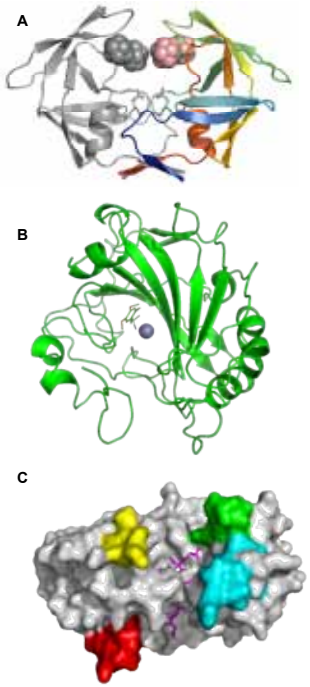

Fig. 2. Examples of crystal structures published in 2009-2010: (A) HIV protease in complex with metallacarborane inhibitor (see reference 2), (B) human carbonic anhydrase II in complex with inhibitor (see reference 1), (C) secreted aspartic protease from *Candida parapsilosis* in complex with pepstatin (see reference 4).

- Ministry of Education, Youth and Sports of the Czech Republic, 1M0505 – Centre of Targeted Therapeutic Drugs, 2005-2011, M. Fábry
- Ministry of Education, Youth and Sports of the Czech Republic, OE 210 – Engineering radionuclide-labelled antibodies, 2006-2009, J. Sedláček
- FP6 EU, 37693 HIV PI RESISTANCE – HIV protease inhibitor resistance by enzyme-substrate coevolution, 2007-2010, J. Sedláček
- GA CR, GA301/07/0600 – Intersubunit complementation studies and characterization of genotype – phenotype correlations in adenylosuccinate lyase deficiency, 2007-2009, J. Brynda
- Ministry of Industry and Trade of the Czech Republic, 2A-2TP1/076 – Generic therapeutic antibodies, 2007-2011, J. Sedláček
- GA CR, GA203/09/0820 – Structure based drug design of specific nucleotidases inhibitors, potentially pharmacologically important compounds, 2009-2013, J. Brynda

1. Gitto R, Agnello S, Ferro S, De Luca L, Vullo D, Brynda J, Mader P, Supuran CT, Chimirri A. Identification of 3,4-dihydroisoquinoline-2[1H]-sulfonamides as potent carbonic anhydrase inhibitors: synthesis, biological evaluation, and enzyme-ligand X-ray studies. **J Med Chem 2010** 53[6]: 2401-2408.
2. Řezáčová P, Pokorná J, Brynda J, Kožíšek M, Cígler P, Lepšík M, Fanfrlík J, Řezáč J, Grantz Šašková K, Sieglová I, Plešek J, Šícha V, Grüner B, Oberwinkler H, Sedláček J, Krausslich HG, Hobza P, Král V, Konvalinka J. Design of HIV protease inhibitors based on inorganic polyhedral metallacarboranes. **J Med Chem 2009** 52[22]: 7132-7141.
3. Šašková KG, Kožíšek M, Řezáčová P, Brynda J, Yashina T, Kagan RM, Konvalinka J. Molecular characterization of clinical isolates of human immunodeficiency virus resistant to the protease inhibitor darunavir. **J Virol 2009** 83[17]: 8810-8818.
4. Dostál J, Brynda J, Hrušková-Heidingsfeldová O, Sieglová I, Pichová I, Řezáčová P. The crystal structure of the secreted aspartic protease 1 from Candida parapsilosis in complex with pepstatin A. **J Struct Biol 2009** 167[2]: 145-152.
5. Řezáčová P, Kožíšek M, Moy SF, Sieglová I, Joachimiak A, Machius M, Otwinowski Z. Crystal structures of the effector-binding domain of repressor Central glycolytic gene Regulator from Bacillus subtilis reveal ligand-induced structural changes upon binding of several glycolytic intermediates. **Mol Microbiol 2008** 69[4]: 895-910.

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Jitka Kredbová / Technician

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Laboratory of Transgenic Models of Diseases

Proteases and their inhibitors, epidermis, liver, IBD, colitis, transgenesis

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Our work is focused on proteinases, especially metalloproteinases that process extracellular matrix (ECM) proteins or release (shed) ligands and their receptors from the cell surface. Interactions between cells and matrix control cell differentiation, survival, migration, and activation via cell surface receptors and adhesion molecules. Especially adhesion molecules sense changes in the composition of extracellular matrix that is affected by proteases and their inhibitors. Balance between these two molecule classes determines if tissues and organ architecture are to be built up or disrupted. Thus, this balance is pivotal for tissue homeostasis and disturbance may lead to development of various pathologies such as cancer, chronic inflammation, or fibrosis.

Our research activities focus on how proteases process ECM-proteins and how these changed matrix proteins (i.e. their fragments) affect the biology of various cell types. These investigations are focused specifically on several research areas: epithelium/epidermis and currently also liver and colon pathogenesis. Inflammatory reactions in these tissues are of our special interest as regulated proteolytic activity in the epidermis and many epithelia is crucial not only to maintenance of the body and organ barriers, but also to regulation of local inflammatory reactions. For instance, we are currently analysing the impact of matrix metalloproteinases 19 and 28 on development of colitis in DSS-induced model using MMP-19- and MMP-28-deficient mice. The exacerbation of the colitis in the knockout mice together with elevated concentrations of pro-inflammatory cytokines derived from colonic tissue suggests that MMP-19 has a role in maintenance of intestinal homeostasis. According to these results we propose a protective effect of MMP-19 during colitis.

To understand development and progression of liver fibrosis and inflammation processes, our research in this area analyses the effects and consequences of metalloproteinase-mediated turnover of extracellular matrix and the release of regulatory molecules from the cellular surfaces, a process that is mediated by shedding proteases.

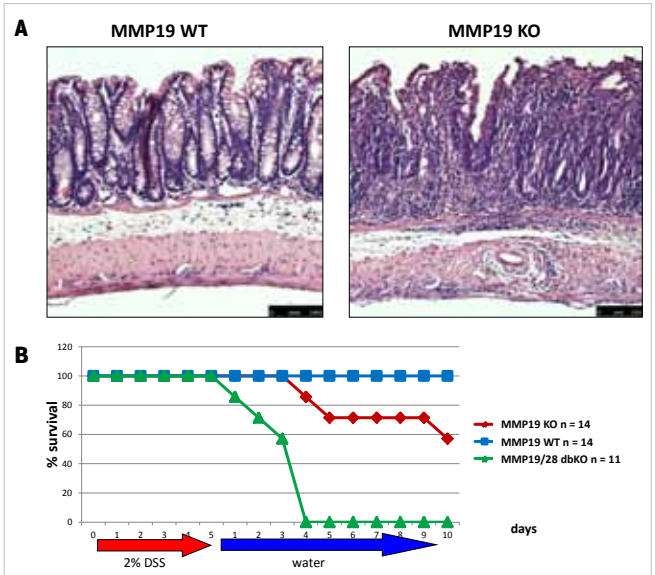

Fig. 1. Matrix metalloproteinases MMP19 and MMP28 play an essential role in the development of colitis. During the course of the experiment the MMP-19-deficient mice loose significantly more weight and show a higher disease activity index than the WT animals: (A) colonic epithelium is severally destroyed and the recovery phase delayed; (B) whereas all WT mice survived treatment and resolving phase of the colitis, only two thirds of MMP-19 KO and none of MMP19/28 dbKO mice are surviving.

- GA CR, GC301/08/J053 – Mouse models for investigation of biological functions of novel Kazal-type protease inhibitors LEKTI-2 and LEKTI-3, 2008-2010, R. Sedláček
- AS CR, IAA500520812 – Investigating the pathologic role of metalloproteinases in liver using transgenic mice with conditional expression of transgenes, 2008-2012, R. Sedláček
- GA CR, GAP305/10/2143 – Generation of mouse models for targeting stellate cells and myofibroblasts in the liver, 2010-2013, R. Sedláček
- GA CR, GAP303/10/2044 – The impact of a liver-specific deficiency of growth factor sheddase ADAM10 on liver development and pathology, 2010-2013, R. Sedláček, M. Jirouškova

1. Muller M, Beck IM, Gadesmann J, Karschuk N, Paschen A, Proksch E, Djonov V, Reiss K, Sedlacek R. MMP19 is upregulated during melanoma progression and increases invasion of melanoma cells. **Mod Pathol 2010** 23(4): 511-521.
2. Lettau I, Hattermann K, Held-Feindt J, Brauer R, Sedlacek R, Mentlein R. Matrix metalloproteinase-19 is highly expressed in astroglial tumors and promotes invasion of glioma cells. **J Neuropathol Exp Neurol 2010** 69(3): 215-223.
3. Beck IM, Ruckert R, Brandt K, Mueller MS, Sadowski T, Brauer R, Schirmacher P, Mentlein R, Sedlacek R. MMP19 is essential for T cell development and T cell-mediated cutaneous immune responses. **PLoS One 2008** 3(6): e2343.

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Laboratory of RNA Biology

RNA splicing, spliceosome formation, alternative splicing, retinitis pigmentosa, nuclear structure

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Our long-term interest is to determine how cells decode information stored in the genome. Information in DNA is fragmented and we study processes and complexes that splice these fragments together and generate meaningful information that is further translated into amino acid sequence in a protein. We focus on molecules called RNAs that serve as a courier between DNA and proteins. However, it appears that RNA does not act as a simple "messenger" but undergoes various changes that significantly change information it carries. Our major aim is to analyse the process called RNA splicing and we mainly focus on variations in splicing among different cells and on assembly of the machinery that catalyses RNA splicing. We also aim to determine why mutations in the splicing machinery cause retinitis pigmentosa, a human genetic disease characterized by photoreceptor cell degeneration. As we study all these interesting processes directly in living cells, we widely employ advanced microscopy techniques (e.g. live cell imaging, FRET, FCS).

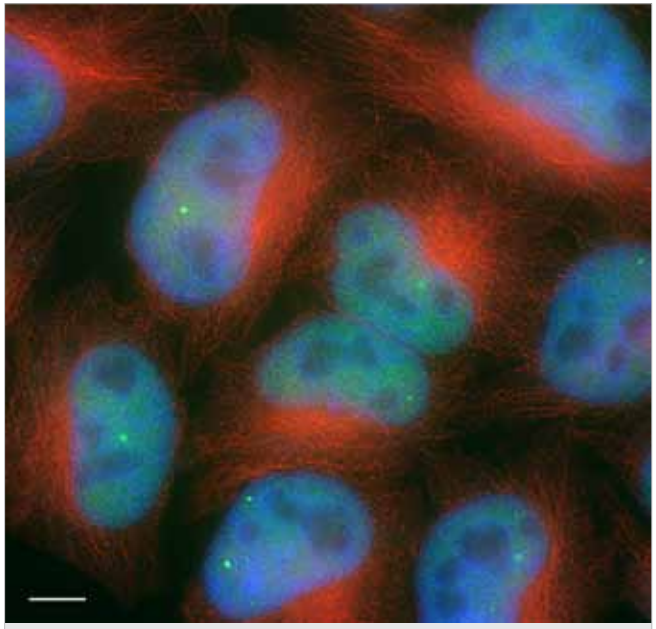

Fig. 1. Cancer cells expressing SART3-GFP protein (green). Cell nuclei stained blue and microtubules in the cytoplasm red.

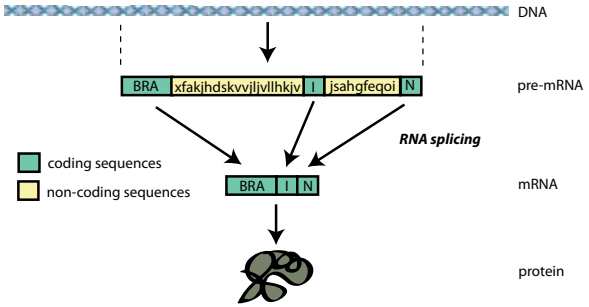

Fig. 2. Information flow from DNA via mRNA to protein.

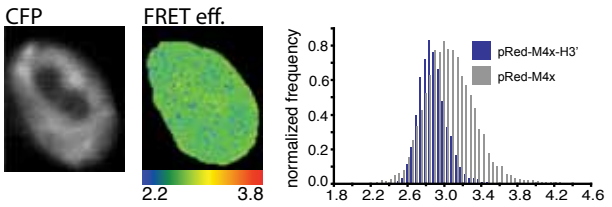

Fig. 3. Visualization of RNA-protein interaction directly in living cells by RB-FRET (Huranova *et al.*, 2009).

- GA CR, GA204/07/0133 – Self-organization principles of non-membrane-bound organelles in eukaryotic cells, 2007-2011, D. Staněk
- AS CR, KAN200520801 – Targeted expression and transport of bioactive molecules, 2008-2012, D. Staněk
- GA CR, GAP305/10/0424 – Regulation of alternative splicing via chromatin acetylation, 2010-2013, D. Staněk
- Max Planck Society – Pre-mRNA splicing and organization of the cell nucleus, 2006-2010, D. Staněk

1. Huranová M, Ivani I, Benda A, Poser I, Brody Y, Hof M, Shav-Tal Y, Neugebauer KM, Staněk D. The differential interaction of snRNPs with pre-mRNA reveals splicing kinetics in living cells. **J Cell Biol 2010** 191(1): 75-86.
2. Huranová M, Jablonski JA, Benda A, Hof M, Staněk D, Caputi M. *In vivo* detection of RNA-binding protein interactions with cognate RNA sequences by fluorescence resonance energy transfer. **RNA 2009** 15(11): 2063-2071.
3. Huranová M, Hnilicová J, Fleischer B, Cvačková Z, Staněk D. A mutation linked to retinitis pigmentosa in HPRP31 causes protein instability and impairs its interactions with spliceosomal snRNPs. **Hum Mol Genet 2009** 18(11): 2014-2023.
4. Cvačková Z, Mašata M, Staněk D, Fidlerová H, Raška I. Chromatin position in human HepG2 cells: although being non-random, significantly changed in daughter cells. **J Struct Biol 2009** 165(2): 107-117.
5. Staněk D, Přidalová-Hnilicová J, Novotný I, Huranová M, Blažíková M, Wen X, Sapra AK, Neugebauer KM. Spliceosomal small nuclear ribonucleoprotein particles repeatedly cycle through Cajal bodies. **Mol Biol Cell 2008** 19(6): 2534-2543.

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Jana Křížová / Technician (until 2010)
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Laboratory of Epigenetic Regulations

RNA degradation, dsRNA, RNAi, miRNA, chromatin

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The zygotic genome activation is the first step in the execution of the genome-encoded program that forms a new organism from a single fertilized cell and it is an essential event in the life of every sexually reproducing organism. The zygotic genome activation is closely associated with formation of pluripotency, i.e. the ability of cells to differentiate into any body cell type. Pluripotency is most studied in two artificial cell types, which maintain pluripotency during *in vitro* culture: embryonic stem cells (ESCs), which are derived from the inner cell mass of the blastocyst, and induced pluripotent stem cells (iPSCs), which form upon reprogramming gene expression in somatic cells with specific pluripotency factors that include transcription factors from the core transcription factor network controlling ESC renewal and pluripotency. A similar network forms in a stepwise manner during the mouse zygotic genome activation, which initiates at the early two-cell stage.

We study reprogramming of oocytes into pluripotent blastomeres of an early embryo (oocyte-to-embryo transition). This model is the natural parallel to the artificial reprogramming of somatic cells into iPSCs. The oocyte-to-embryo transition, however, is distinct. It is a unidirectional transient process executed by cytoplasmic factors, as demonstrated by animal cloning by nuclear transfer. Our primary research interest is in post-transcriptional mechanisms underlying oocyte-to-embryo transition. These mechanisms include control of maternal mRNA stability, small regulatory RNAs in miRNA and RNAi pathways, and production of maternal transcription factors, which will control gene expression in the embryo. Our goal is to understand how control of gene expression creates developmental competence *in vivo*.

Research of pluripotency is eminent for medicine and biotechnology where pluripotency plays a role in an ever-growing number of applications. Understanding control of the oocyte-to-embryo transition will provide original insights into stem cell biology and will likely contribute to efficient and safe production of pluripotent stem cells, efficient cloning technologies, informative prenatal diagnostics, and understanding of pathology of sterility and developmental defects.

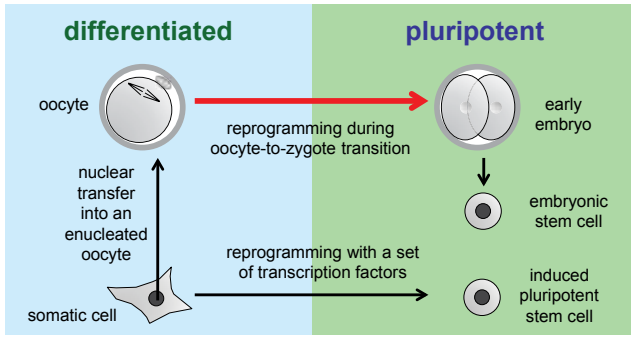


Fig. 1. Oocyte-to-zygote transition is a unique model for studying pluripotency. The mammalian oocyte is a highly specialized cell, whose cytoplasm is capable of reprogramming a genome to initiate development of a new organism. The blastomeres of the 2-cell embryo are totipotent as they can give a rise to embryonic and extraembryonic tissues. The pluripotent embryonic stem cells, which have potential to give a rise to any body cell type, are derived from the blastocyst, the final preimplantation embryo stage carrying the first defined cell lineages.

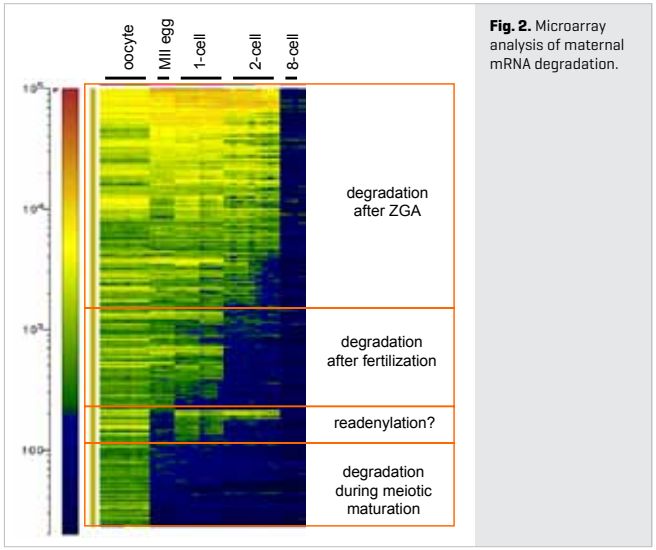


Fig. 2. Microarray analysis of maternal mRNA degradation.

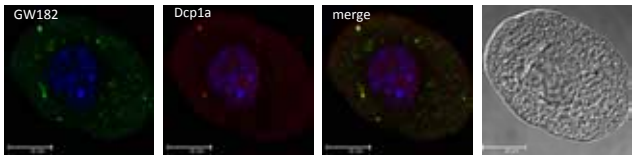
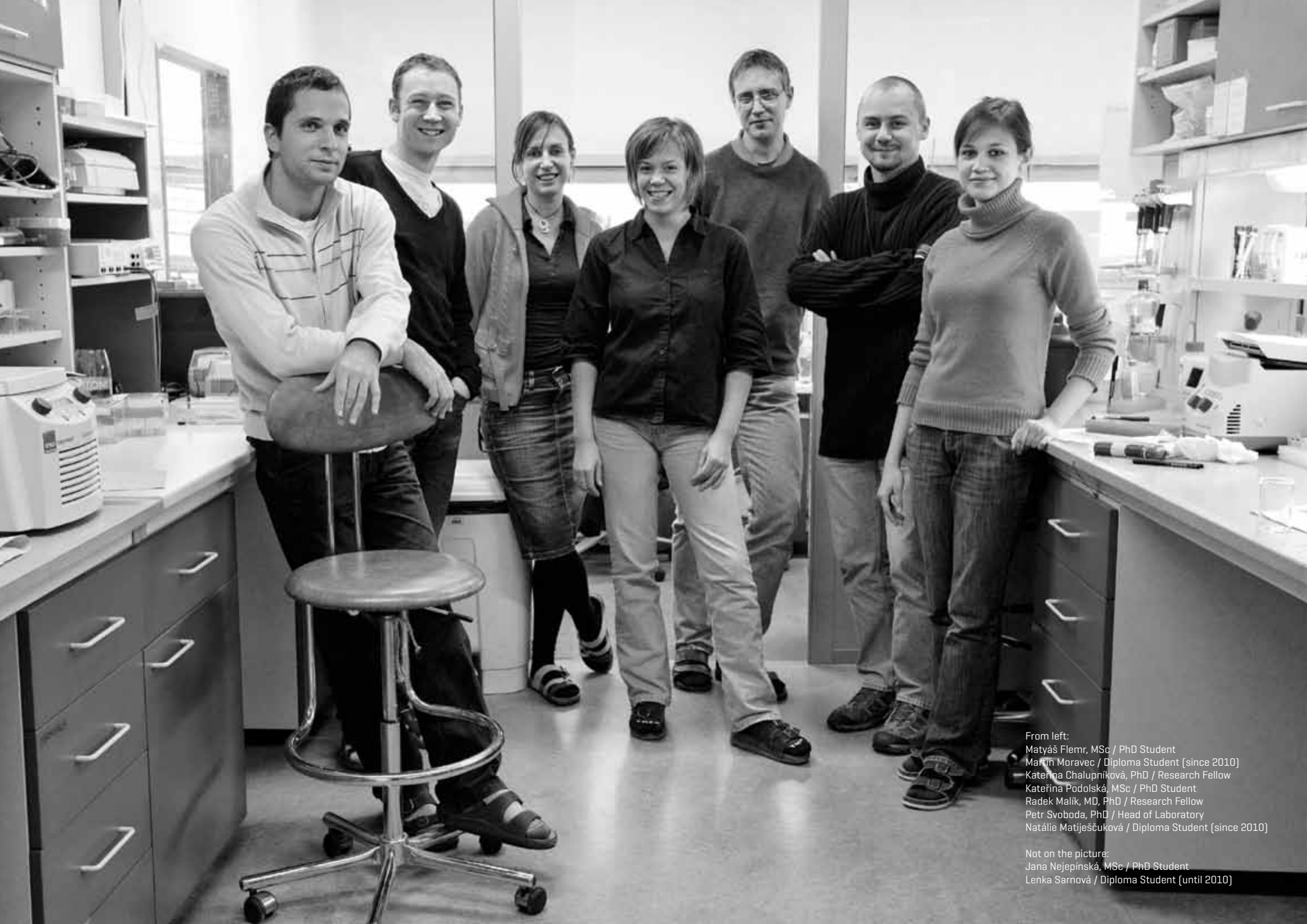


Fig. 3. Colocalization of p-body components in meiotically incompetent oocytes. P-bodies are centres of mRNA metabolism, including degradation and storage.

- EMBO, 1483 – EMBO Installation Grant, 2007-2011, P. Svoboda
- Ministry of Education, Youth and Sports of the Czech Republic, ME09039 – Role of posttranslational mechanisms in reprogramming mouse oocytes to pluripotent cells, 2009-2012, P. Svoboda
- GA CR, GA204/09/0085 – RNA silencing and long dsRNA in mammalian cells, 2009-2013, P. Svoboda
- GA CR, GAP305/10/2215 – Control of chromatin and pluripotency by microRNAs, 2010-2013, P. Svoboda
- GA Charles University, 18110 – Development and characterization of microRNA pathway inhibitor, 2010-2012, K. Podolská

1. Svoboda P, Flemr M. The role of miRNAs and endogenous siRNAs in maternal-to-zygotic reprogramming and the establishment of pluripotency. **EMBO Rep 2010** 11(8): 590-597.
2. Flemr M, Svoboda P. Ribonucleoprotein localization in mouse oocytes. **Methods 2011** 53(2): 136-141.
3. Ma J, Flemr M, Stein P, Berninger P, Malik R, Zavolan M, Svoboda P, Schultz RM. MicroRNA activity is suppressed in mouse oocytes. **Curr Biol 2010** 20(3): 265-270.
4. Flemr M, Ma J, Schultz RM, Svoboda P. P-Body Loss Is concomitant with formation of a messenger RNA storage domain in mouse oocytes. **Biol Reprod** 2010 82(5): 1008-1017.
5. Sinkkonen L, Hugenschmidt T, Berninger P, Gaidatzis D, Mohn F, Artus-Revel CG, Zavolan M, Svoboda P, Filipowicz W. MicroRNAs control de novo DNA methylation through regulation of transcriptional repressors in mouse embryonic stem cells. **Nat Struct Mol Biol 2008** 15(3): 259-267.



From left:
Matyáš Flemr, MSc / PhD Student
Martin Moravec / Diploma Student (since 2010)
Kateřina Chalupníková, PhD / Research Fellow
Kateřina Podolská, MSc / PhD Student
Radek Malík, MD, PhD / Research Fellow
Petr Svoboda, PhD / Head of Laboratory
Natálie Matiješčuková / Diploma Student (since 2010)

Not on the picture:
Jana Nejepínská, MSc / PhD Student
Lenka Sarnová / Diploma Student (until 2010)

Laboratory of Genomics and Bioinformatics

Genomics, next-generation sequencing, genome, microarray transcriptional profiling, cancer

Čestmír Vlček

cestmir.vlcek@img.cas.cz

Our laboratory was amongst the first to complete genome projects. Information generated in these projects was used in evolutionary studies and recently also in biotechnological applications. Using the 454 next-generation sequencing facility (GS FLX/Titanium), we characterize genomes of different species and metagenomic samples. To understand the evolution of eukaryotes and the developmental processes that they regulate, it is necessary to analyse their genomes. Single-cell eukaryotes with their branching close to the root of the evolutionary tree are the best candidates for genome studies. The availability of the genomic sequences will allow inferences to be made about the gene complement of the common eukaryotic ancestor. The genome projects under way are that of *Mastigamoeba balamuthi* and *Diplonema papillatum*. The metagenomic analyses of environmental samples and unculturable microbes are under way, too.

A second major project of our group is directed towards identification of markers specific for head and neck cancer tissue with potential applications in medical diagnosis. We use the Illumina microarray chip technique for detection of appropriate gene sets that are upregulated in this cancer disease. The found markers could identify the disease subtype and so help to aim the treatment.

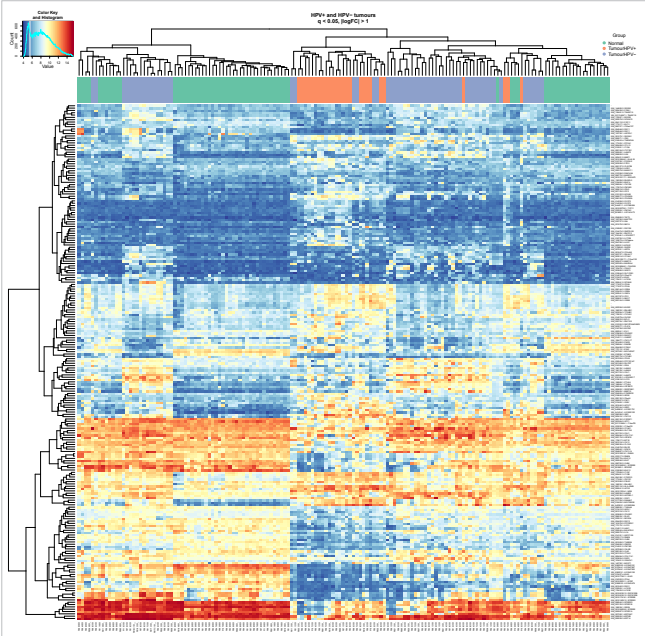


Fig. 1. Graphical presentation of differences in expression of genes. The samples have been taken from head and neck squamous cell cancer patients with and without human papilloma virus infection.

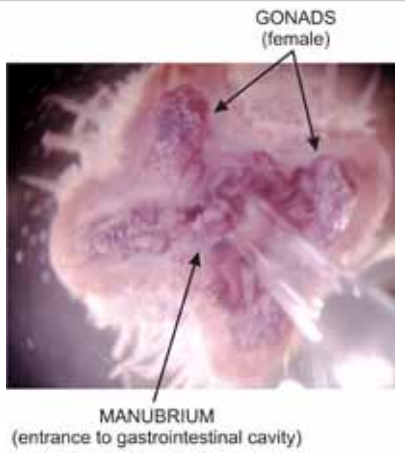
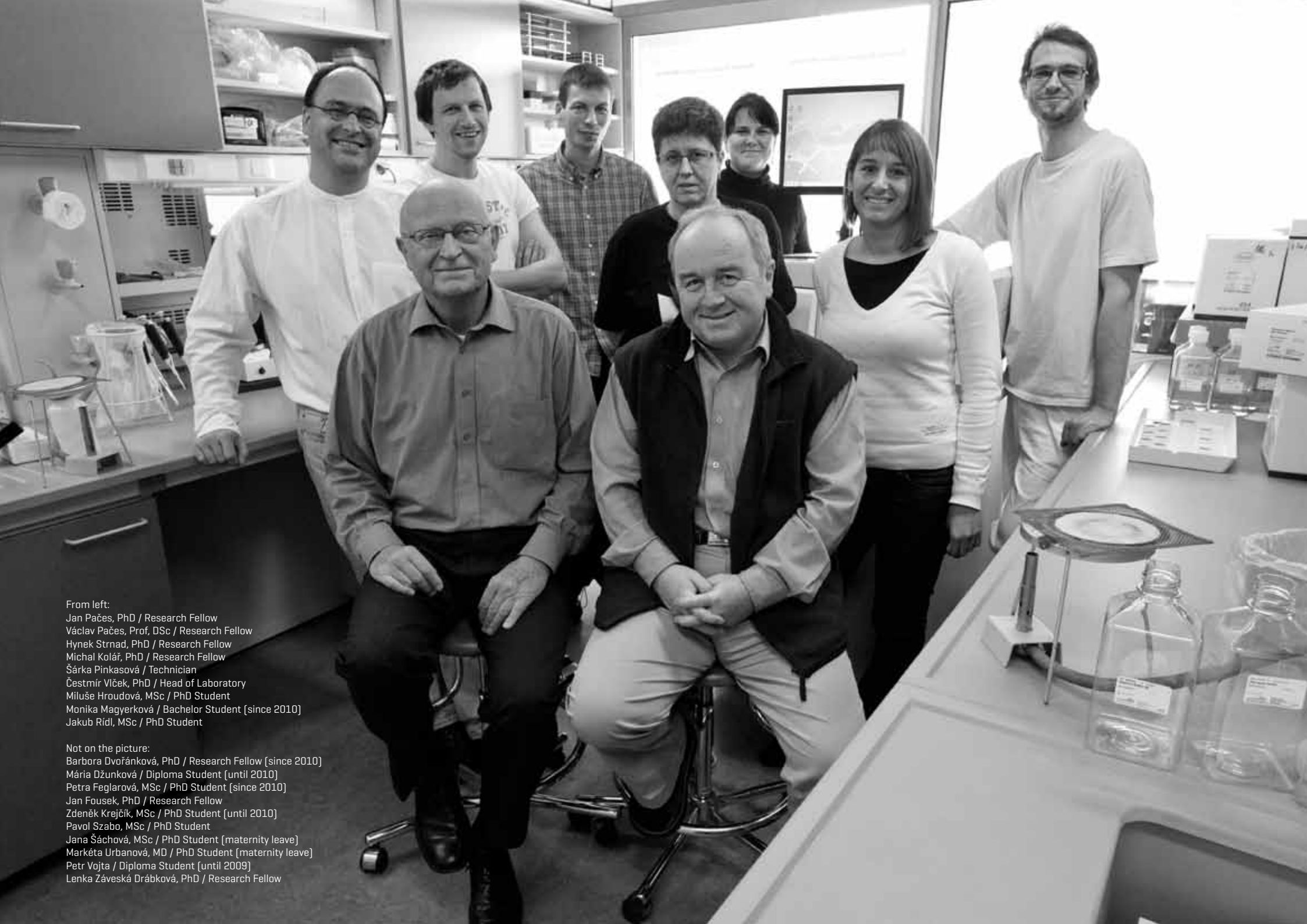


Fig. 2. Tissue-specific expression pattern of csSix4/5A transcription factor visualized (dark violet) by whole-amount *in situ* hybridization of adult jellyfish *Craspedacusta sowerbyi* (freshwater hydrozoan cnidarian). Expression is localized in gonads (germ cells, production of oocytes) and at the margin of manubrium (nerve cells – mechanosensors, feeding).

Fig. 3. Next-generation sequencer GS FLX (Roche).

- Ministry of Education, Youth and Sports of the Czech Republic, 1M0520 – Center for Applied Genomics, 2005-2011, V. Pačes
- Ministry of Education, Youth and Sports of the Czech Republic, 2B06106 – Novel genomic and biotechlological approaches in molecular oncology: a way to the early diagnostics and targeted therapy, 2006-2011, Č. Vlček
- Ministry of Education, Youth and Sports of the Czech Republic, 2B08031 – Metagenomics and bioinformatics as a basis for preparation of effective approaches, preparation and characterization of microorganisms and their consortia for utilization in bioremediation, 2008-2011, J. Pačes
- GA CR, GCP305/10/J052 – Functional analysis of endogenous retroviral elements in human genome: possible association with cancers, 2010-2012, J. Pačes

1. Strnad H, Lacina L, Kolář M, Čada Z, Vlček C, Dvořánková B, Betka J, Plzák J, Chovanec M, Šáchová J, Valach J, Urbanová M, Smetana K Jr. Head and neck squamous cancer stromal fibroblasts produce growth factors influencing phenotype of normal human keratinocytes. **Histochem Cell Biol 2010** 133(2): 201-211.
2. Mihola O, Trachtulec Z, Vlcek C, Schimenti JC, Forejt J. A mouse speciation gene encodes a meiotic histone H3 methyltransferase. **Science 2009** 323(5912): 373-375.
3. Kozmik Z, Swamynathan SK, Ruzickova J, Jonasova K, Paces V, Vlcek C, Piatigorsky J. Cubozoan crystallins: evidence for convergent evolution of pax regulatory sequences. **Evol Dev 2008** 10(1): 52-61.
4. Jencova V, Strnad H, Chodora Z, Ulbrich P, Vlcek C, Hickey WJ, Paces V. Nucleotide sequence, organization and characterization of the (halo)aromatic acid catabolic plasmid pA81 from Achromobacter xylosoxidans A8. **Res Microbiol 2008** 159(2): 118-127.
5. Kozmik Z, Ruzickova J, Jonasova K, Matsumoto Y, Vopalensky P, Kozmikova I, Strnad H, Kawamura S, Piatigorsky J, Paces V, Vlcek C. Assembly of the cnidarian camera-type eye from vertebrate-like components. **Proc Natl Acad Sci USA 2008** 105(26): 8989-8993.



From left:
Jan Pačes, PhD / Research Fellow
Václav Pačes, Prof, DSc / Research Fellow
Hynek Strnad, PhD / Research Fellow
Michal Kolář, PhD / Research Fellow
Šárka Pinkasová / Technician
Čestmír Vlček, PhD / Head of Laboratory
Miluše Hroudová, MSc / PhD Student
Monika Magyerková / Bachelor Student (since 2010)
Jakub Rídl, MSc / PhD Student

Not on the picture:
Barbora Dvořánková, PhD / Research Fellow (since 2010)
Mária Džunková / Diploma Student (until 2010)
Petra Feglarová, MSc / PhD Student (since 2010)
Jan Fousek, PhD / Research Fellow
Zdeněk Krejčík, MSc / PhD Student (until 2010)
Pavol Szabo, MSc / PhD Student
Jana Šáchová, MSc / PhD Student (maternity leave)
Markéta Urbanová, MD / PhD Student (maternity leave)
Petr Vojta / Diploma Student (until 2009)
Lenka Záveská Drábková, PhD / Research Fellow

Information Technologies

Petr Divina

petr.divina@img.cas.cz

The IT department provides a range of information technology services to support various needs within the Institute. The main tasks include the administration of LAN and wireless network in the domain img.cas.cz, administration of institutional servers (DNS, mail, web) and storage area network (SAN) infrastructure, and performing data backup and archiving. The critical information technology equipment is housed in modern data centre rooms with controlled air-conditioning, uninterrupted power supply, temperature and humidity monitoring, and a fire protection system. The network security is assured by a firewall appliance that allows secure remote access to the computer network (VPN) and anti-spam plus anti-virus solutions, both on the server side and on the user computers. On a daily basis, the IT department ensures the installation and registration of computers and printers to the computer network, hardware purchase and consultancy, and support to users of Windows and Macintosh platforms. For commonly used software at the Institute, the volume and site licensing options are negotiated. Special support is provided to other technical and scientific departments, e.g. developing simple websites and on-line tools, maintaining dedicated databases, such as animal tracking system. The IT department also operates the audio-visual equipment in the conference hall and provides computers for courses and conferences organized at the Institute.

Main data centre room

Computer classroom

Firewall and central switch

Tape library and disk storage

From left:
Tomáš Drexler
Petr Janků, MSc
Petr Divina, PhD / Head
Jakub Šimon
Michal Rolník
Michal Kůs
Pavel Dvořák

Not on the picture:
Miroslav Indra, PhD

Genomics and Bioinformatics

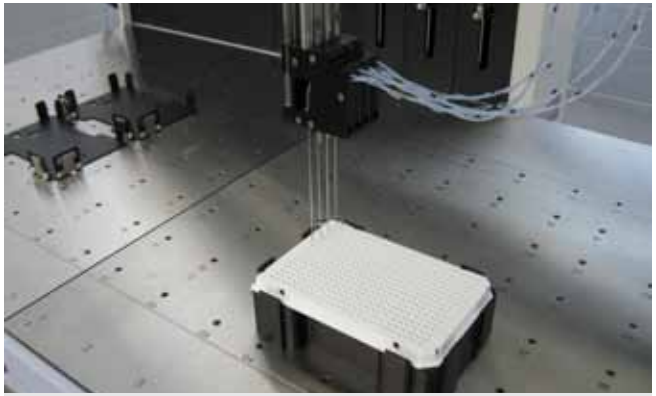
Hynek Strnad

hynek.strnad@img.cas.cz

The facility was established in late 2005 after the purchase of the Affymetrix GeneChip System and was initially operated by the staff from the Laboratory of Mouse Molecular Genetics. Since January 2007, it has become an independent unit which provides full chip microarray services, real-time quantitative PCR service and high-throughput methods using the robotic equipment. The services are provided not only to the research groups at the Institute of Molecular Genetics, but also to other academic institutions in the Czech Republic as well as abroad. The core facility is equipped with two microarray platforms: Affymetrix GeneChip System and Illumina BeadStation 500, real-time PCR cycler Roche LC480, JANUS robots and EnVision Plate Reader from PerkinElmer, and also with instruments for assessment of quality and quantity of processed samples (spectrophotometer Nanodrop and capillary electrophoresis Agilent Bioanalyzer 2100). The facility represents one of the European certified Affymetrix core labs.

Illumina BeadArray Reader

Affymetrix GeneChip Scanner



PerkinElmer Janus

Agilent Bioanalyzer

From left:
Hynek Strnad, PhD / Head (since 2009)
Jitka Dubská, MSc (since 2009)
Veronika Klatovská, MSc
Martina Chmelíková, MSc

Not on the picture:
Robert Ivánek, MSc (until 2009)

Monoclonal Antibodies and Cryobank

Dobromila Matějková

dobromila.matejkova@img.cas.cz

Monoclonal Antibodies Facility

The facility provides preparation of mouse monoclonal antibodies including immunization using a particular immunization protocol, ELISA testing of production of specific antibodies, cloning of selected samples, freezing of cryobank samples, cultivation of cell culture supernatants, or preparation of ascitic fluid from selected clones and isotype determination of the produced antibody. Further services comprise testing of cell culture supernatants for the presence of mycoplasms and freezing of cell line banks and hybridomas.

Cryobank

The cryobank serves for long-term storage of samples in liquid nitrogen. The current cryobank capacity is 320,000 samples, with further possible extension. The cryobank stores cell lines, hybridomas, mouse sperm and mouse embryos in liquid nitrogen or its vapours. The storage containers (LABS40K – Taylor-Wharton and 24K) are connected to the exterior liquid nitrogen container for 6,000 litres and supplied automatically. The entire cryobank system is secured by a backup energy source in case of power failure. All operations, diagnostics and monitoring of the level of liquid nitrogen in the storage containers are fully automated and controlled. Parameters (temperature, humidity, O_2 concentration) and safety both in the cryobank and in the individual storage containers are followed by the monitoring system with GSM and web interface outputs.

Monoclonal antibodies laboratory

Storage of samples in liquid nitrogen

Tissue culture incubator for preparation of monoclonal antibodies

Liquid nitrogen storage vessels

From left:
Hana Korábová
Dobromila Matějková, MSc / Head

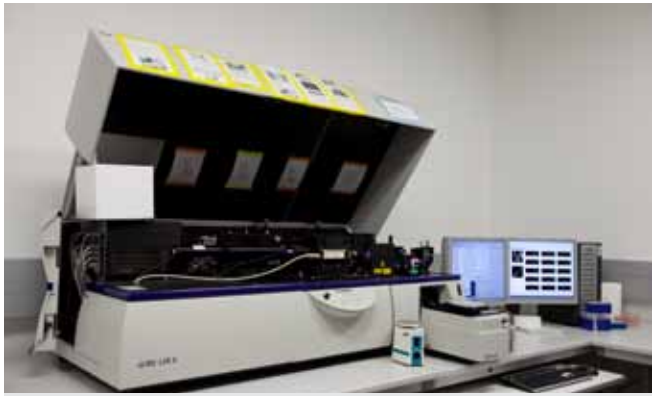
Not on the picture:
Šárka Šilhánková (until 2009)

Flow Cytometry and Light Microscopy

Ondrej Horváth

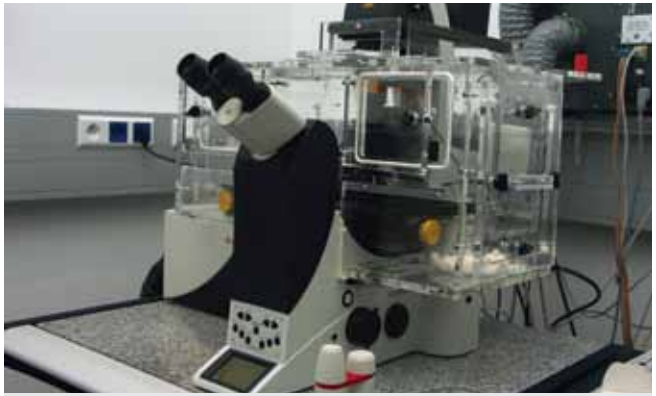
ondrej.horvath@img.cas.cz

The facility provides methodological and instrumentation background for flow cytometric and fluorescence microscopy techniques. At present, the facility is equipped with two cytometers – BD FACSCalibur and BD LSRII. The LSRII instrument is a four-laser (405, 488, 561 and 633-nm) instrument with 14 fluorescence detectors. A large set of dichroic mirrors and bandpath filters are available in the laboratory, making this instrument very flexible and capable to cover most of the flow cytometry applications. Both cytometers are equipped with the HTS loader for high-throughput analysis of samples directly from 96- or 384-well plates. The facility is also equipped with an AutoMACS Pro (Miltenyi Biotec) magnetic separator for automatic rapid sorting of cells, as well as cell culture facilities. The facility is running three microscopes: laser scanning confocal microscope with superfast scanner (Leica TCS SP5 AOBS TANDEM), Leica inverted fluorescent microscope with TIRF illumination (Leica), wide-field inverted fluorescence microscope with laser photomanipulation (DeltaVision Core). This state-of-art instrumentation allows facility users to use a wide range of microscopy techniques including FRET, FRAP, time-lapse experiments, membrane studies, vesicle transport studies, etc. Several offline analysis workstations are also available in the facility, for analysis of flow cytometric (FlowJo) and image data (SoftWorx Suite, Imaris, LAS AF, Huygens, ImageJ). In the near future the laboratory will be equipped with a high-speed cell sorter.



LSRII flow cytometer

DeltaVision Core deconvolution microscope with laser photomanipulation



SP5 TCS AOBS Tandem confocal microscope

Leica TIRF microscope

From left:
Ondrej Horváth, MD / Head
Zdeněk Cimburek

Media and Glass Washing

Hana Marxová

hana.marxova@img.cas.cz

The service unit offers preparation of tissue culture media and solutions (ranging from redistilled deionized water and PBS through media such as RPMI, MEM varieties, HBSS, trypsin, to custom-made solutions), preparation of bacteriology media and plates (clear and with selection agents), sterilization of solutions and material (steam sterilization, filtration of various grades), distribution of FBS, transfection agents, glass and plastic washing, decontamination of GMO and other hazardous waste (annual volume about 5,000 kg), organization of working cloth washing (more than 4,000 items per year).

Preparation of sterilization of glass pipettes

Steam sterilization of solutions

Hot-air sterilization

Sterile filtration

From left:
Lenka Alferiová
Jitka Škopová
Stanislava Bendová
Hana Marxová / Head

Not on the picture:
Miluše Alferiová (until 2009)

Transgenic Unit

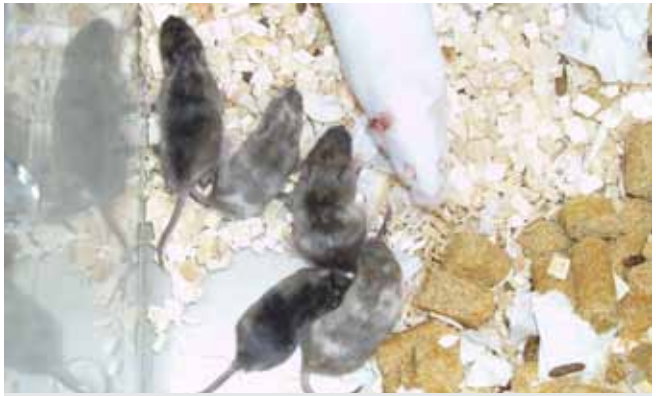
Radislav Sedláček

radislav.sedlacek@img.cas.cz

Genetically modified mouse models have become a key tool in basic and biomedical research. The ability to engineer the mouse genome has greatly transformed biomedical research in the last decade. Crucial for this technology is the ability to control the expression of genes of interest. It is possible to increase or decrease gene expression, or eliminate the expression of a gene completely. Transgenic and gene knock-out/knock-in technologies have become important experimental tools for assigning functions to genes at the level of whole complexity of organism, creating models of genetic disorders, evaluating effects of potential therapeutic targets and drugs, and thus helping to answer fundamental issues in basic and applied research.

The Transgenic Unit at the Institute of Molecular Genetics (TgU) was established under the direction of Assoc Prof Radislav Sedláček in 2008/2009 to offer the research community at IMG and other cooperating institutions the opportunity to study the function of individual genes using mouse models.

Our services include pronuclear microinjection of DNA constructs into mouse zygotes for the production of transgenic founders; microinjection of targeted ES cell lines into morulas (8-stage cell embryo) to produce chimeric mice; mouse archiving (cryopreservation of embryos and sperm); and recovery of live mice from cryopreserved embryos and sperm, analysis of sperm viability, re-derivation of mouse strains and lines, and others (see also our web page at **http://tgunit.img.cas.cz/**). We also provide consultation and assistance services, and information on the design and use of genetically modified transgenic mice. All of our services are also available to external institutions and researches irrespective whether they are from academic or profit



Foster mother with chimeric pups

Implantation of embryos under SPF conditions

Pronuclear injection

Transgenic core facility

From left:
Sandra Potyšová, MSc [since 2010]
Radislav Sedláček, Assoc Prof, PhD / Head
Veronika Libová, MSc [since 2009]
Inken Maria Beck, PhD

Not on the picture:
Irena Placerová, MSc
Lenka Michalčíková, MSc [until 2010]
Jana Ježková, MSc [until 2009]

Animal Facility (Mice)

Jan Honetschläger

jan.honetschlager@img.cas.cz

The IMG animal (mouse) facility has recently been housed in three buildings on the Krč campus and is accredited for work with genetically modified animals, with total capacity up to 8,000 cages. To date, we have available about 160 mouse strains; most of them are unique. All animals are housed under standard pathogen-free conditions required for high-quality research. We built a new quarantine with a capacity up to 400 cages, allowing us to import animals from cooperating institutes more easily. The animal facility also hosts the Transgenic Unit, which produces various types of transgenic and gene knock-out mice and performs re-derivation from the quarantine. Another building of the animal facility that was renovated in 2010 accommodates the experimental space for poultry. It also houses instruments for X-ray irradiation and sonography, both available to the entire Campus of Biomedical Institutes of the Academy of Sciences.

Animal facility building

X-ray instrument T-200

IVC rack and changing station

BALB/c mice

From left:
Alena Babanská (since 2010)
Renáta Cihelková
Zuzana Novotná, MSc
Jarmila Krestová
Dagmar Čermáková
Soňa Hellerová
Daniela Vorlová (since 2010)
Michaela Lišáková
Alena Zachardová
Jan Honetschläger, DVM / Head
Monika Novotná
Kamila Malá
Miloslava Kudličová
Zuzana Bakešová
Renáta Koubová (since 2010)
Pavla Kameníková

Not on the picture:
Jana Kopkanová, MSc (maternity leave)
Lenka Rysslová (maternity leave)
Kateřina Ševčíková (maternity leave)

Animal Facility (Chicken)

Martina Ješátková

martina.jesatkova@img.cas.cz

This facility is located in the village Koleč, north of Prague, about 45 km from the main Campus of Biomedical Institutes in Prague-Krč. It mainly takes care of breeding genetically defined inbred, congenic and outbred chicken lines (and one duck line). The facility produces eggs, embrya and chickens for several research groups focusing on chicken models. Until the experimental laboratories for work with chicken are reconstructed on the Prague-Krč Campus, the facility is also used for experimental laboratory work.

1-day-old chicks

Brown Leghorn cocks (outbred line)

Brown Leghorn hen and cock

White Leghorn cocks (inbred line)

From left:
Petra Faloutová
Jaroslava Strnadelová
Martina Ješátková, MSc / Head
Jitka Dvořáková
Alena Eisensteinová
Radomíra Skoková
Alena Porazilová
Miloslava Vilhelmová, PhD

Not on the picture:
Eva Bernášková
Ladislava Hachová (until 2010)
Zdena Koptová
Kamila Thunová
Miloslava Vaverková (until 2009)
Jaroslava Vlasáková

Building Maintenance

Miroslav Heyduk

miroslav.heyduk@img.cas.cz

Entrance hall

Milan Hašek Auditorium

Central staircase

Cafeteria

Meeting point

View from the park

From left:
Jana Boučková
Miroslav Heyduk, MSc / Head (since 2010)
Dana Macková

Not on the picture:
Petr Blahout
Tomáš Němec, Bc (until 2010)

Office of the Director

Šárka Takáčová

sarka.takacova@img.cas.cz

From left:
Kateřina Sedláčková
Leona Krausová
Šárka Takáčová, MSc / Head
Gabriela Marešová
Zdeňka Schuhová
Jiří Jonák, Prof, MD, DSc

Finances and Administration

Renata Schönová

renata.schonova@img.cas.cz

From left:
Michal Švestka
Hana Nezbedová
Milena Petríková
Jaroslava Samohylová
Miloslava Šnajbergová
Jitka Emanuelová
Kateřina Drastilová
Renata Schönová / Head
Hana Švestková
Ivana Brabencová

Not on the picture:
Milena Dobrá (until 2009)
Zdeňka Sokolová (until 2009)
Emilie Štorchová
Ludvík Vítek (until 2010)

BIOCEV Division

Jan Rajnoch

jan.rajnoch@img.cas.cz

The BIOCEV division of IMG was established in September 2009. Its main task is to ensure, in terms of organization, the preparation and implementation of the project of a new research centre - **Bio**technology and Biomedicine **Ce**ntre of Academy of Sciences and Charles University in **V**estec – BIOCEV. BIOCEV is a joint project of six institutes of the Academy of Sciences of the Czech Republic (Institute of Molecular Genetics, Institute of Microbiology, Institute of Physiology, Institute of Experimental Medicine, Institute of Biotechnology, and Institute of Macromolecular Chemistry) and Charles University in Prague, represented here by two faculties (Faculty of Science and First Faculty of Medicine), and is prepared with the ambition of creating a European centre of excellence.

The project builds upon three pillars of the knowledge triangle. The **research programme**, which provides scientific outputs of highest quality, stands above all. It involves five fundamental areas: Functional Genomics, Cellular Biology and Virology, Structural Biology and Protein Engineering, Biomaterials and Tissue Engineering, and Development of Diagnostic and Therapeutic Procedures. This scientific programme will be supported by several top-quality core facilities.

Transfer of research results into practice represents the second pillar of BIOCEV. The centre will focus on intensive collaboration with the commercial sphere and will support preservation of intellectual property and its further utilization.

The third pillar is **teaching and education** namely of PhD students, which will be achieved within current study programmes of Charles University and by newly accredited programmes in biotechnology and biomedicine. In addition, BIOCEV will organize and facilitate specialized international courses for Czech and international students and young scientists. The new centre will also offer training of business employees in advanced biotechnology methods.

BIOCEV has entered two networks of European consortia of ESFRI (European Strategy Forum on Research Infrastructures) - EuroBioImaging and INFRAFRONTIER. This integration into the European Research Area even before establishment of BIOCEV itself marks the quality of the future research centre.

The new centre will be built in the municipality of Vestec, Central Bohemia and will accommodate up to 600 employees and 250 Master and PhD students. Estimated costs for construction amount to 92 mil EUR. Funding will be provided by the European Regional Development Fund within the Operational Programme Research and Development for Innovations. The construction should start in the middle of 2011, its operation then late in 2013. For more information visit **http://www.biocev.eu/**.

Visualization of the Centre

From left:
Romana Maštalířová, MSc (since 2009)
Jan Rajnoch, MD / Head
Ivana Sýkorová, PhD (since 2009)
Božena Šléglová (since 2010)

Not on the picture:
Jana Bartušková, MA (02–07/2010)

Council of the IMG

The Council of the Institute serves as an advisory authority to the Director and decides on essential scientific and organizational issues. Its members are appointed by election.

Jiří Forejt, Prof, DSc
[IMG]
Chairman

Jiří Hejnar, PhD
[IMG]
Vice-Chairman

Petr Dráber, DSc
[IMG]

Michal Dvořák, PhD
[IMG]

Václav Hořejší, Prof, PhD
[IMG]

Pavel Hozák, Prof, DSc
[IMG]

Vladimír Kořínek, PhD
[IMG]

Peter Šebo, PhD
[Institute of Biotechnology of the ASCR]

Vladimír Havlíček, Assoc Prof, PhD
[Institute of Microbiology of the ASCR]

Marek Moša, PhD
[SEVAPHARMA a.s.]

Marek Jindra, Assoc Prof, PhD
[Biology Centre of the ASCR]

Jan Tachezy, Prof, PhD
[Faculty of Science, Charles University]

Supervisory Board

The main task of the Supervisory Board is to monitor the financial and legal matters connected with the Institute administration. Its members have been selected by the Academy of Sciences from Academy and business sphere representatives.

Miroslav Flieger, PhD
Chairman
(Academy Council of the ASCR)

Jiří Špička, MSc, MBA
Vice-Chairman
(Deputy Director, IMG)

Martin Fusek, Assoc Prof, PhD
(IOCB TTO s.r.o.)

Jaroslav Kuneš, DSc
(Institute of Physiology of the ASCR)

David Štůla, MA
(Lawyer)

Publications 2009-2010

ANDĚRA

- Anděra L. Signalling activated by the death receptors of the TNRF family. **Biomed Pap 2009** 153(3): 173-180.
- Klener P Jr, Leahomschi S, Molinsky J, Simonova T, Nečas E, Gašová Z, Čermák J, Doležalová L, Anděra L, Živný J. TRAIL-induced apoptosis of HL60 leukemia cells: Two distinct phenotypes of acquired TRAIL resistance that are accompanied with resistance to TNFα but not to idarubicin and cytarabine. **Blood Cells Mol Dis 2009** 42(1): 77-84.
- Klíma M, Zájedová J, Doubravská L, Anděra L. Functional analysis of the posttranslational modifications of the death receptor 6. **Biochim Biophys Acta-Mol Cell Res 2009** 1793(10): 1579-1587.
- Morrison BJ, Anděra L, Reynolds BA, Ralph SJ, Neužil J. Future use of mitocans against tumour-initiating cells? **Mol Nutr Food Res 2009** 53(1): 147-153.
- Oikonomou E, Kosmidou V, Katseli A, Kothonidis K, Mourtzoukou D, Kontogeorgos G, Anděra L, Zografos G, Pintzas A. TRAIL receptor upregulation and the implication of KRAS/BRAF mutations in human colon cancer tumours. **Int J Cancer 2009** 125(9): 2127-2135.
- Živný J, Klener P, Pytlík R, Anděra L. The role of apoptosis in cancer development and treatment: focusing on the development and treatment of hematologic malignancies. **Curr Pharm Des 2010** 16(1): 11-33.

BARTEK

- Bartek J, Hodný Z. SUMO boosts the DNA damage response barrier against cancer. **Cancer Cell 2010** 17(1): 9-11.
- Blažková H, Krejčíková K, Moudrý P, Frisan T, Hodný Z, Bartek J. Bacterial intoxication evokes cellular senescence with persistent DNA damage and cytokine signalling. **J Cell Mol Med 2009** 14(1-2): 357-367.
- Hodný Z, Hubáčková S, Bartek J. Cytokines shape chemotherapy-induced and 'bystander' senescence. **Aging 2010** 2(7): 375-376.
- Hodny Z, Mistrik M, Bartek J. HERCulean giant orchestrates ubiquitin-mediated signaling on damaged chromosomes. **Cell Cycle 2010** 9(7): 1227-1228.
- Hubáčková S, Nováková Z, Krejčíková K, Košař M, Dobrovolná J, Dušková P, Hanzlíková H, Vančurová M, Barath P, Bartek J, Hodný Z. Regulation of the PML tumor suppressor in drug-induced senescence of human normal and cancer cells by JAK/STAT-mediated signaling. **Cell Cycle 2010** 9(15): 3085-3099.
- Jackson SP, Bartek J. The DNA-damage response in human biology and disease. **Nature 2009** 461(7267): 1071-1078.
- Macurek L, Lindqvist A, Voets O, Kool J, Vos HR, Medema RH. Wip1 phosphatase is associated with chromatin and dephosphorylates γH2AX to promote checkpoint inhibition. **Oncogene 2010** 29 (15): 2281-2291.
- Nováková Z, Hubáčková S, Košař M, Janderová-Rossmeislová L, Dobrovolná J, Vašicová P, Vančurová M, Hořejší Z, Hozák P, Bartek J, Hodný Z. Cytokine expression and signaling in drug-induced cellular senescence. **Oncogene 2010** 29(2): 273-284.

BARTŮNĚK

- Eignerová B, Sedlák D, Dračínský M, Bartůněk P, Kotora M. Synthesis and biochemical characterization of a series of 17α-perfluoroalkylated estradiols as selective ligands for estrogen receptor α. **J Med Chem 2010** 53 (19): 6947-6953.
- Gamper I, Koh K-R, Ruau D, Ullrich K, Bartůňková J, Piroth D, Hacker C, Bartůněk P, Zenke M. GAR22: A novel target gene of thyroid hormone receptor causes growth inhibition in human erythroid cells. **Exp Hematol 2009** 37(5): 539-548.
- Sedlák D, Novák P, Kotora M, Bartůněk P. Synthesis and evaluation of 17α-arylestradiols as ligands for estrogen receptor α and β. **J Med Chem 2010** 53 (10): 4290-4294.
- Stachura DL, Reyes JR, Bartůněk P, Paw BH, Zon LI, Traver D. Zebrafish kidney stromal cell lines support multilineage hematopoiesis. **Blood 2009** 114(2): 279-289.
- Zíková M, Corlett A, Bendová Z, Pajer P, Bartůněk P. DISP3, a sterol-sensing domain-containing protein that links thyroid hormone action and cholesterol metabolism. **Mol Endocrinol 2009** 23(4): 520-528.

BLAHOŠ

- Pávek P, Pospěchová K, Švecová L, Syrová Z, Stejskalová L, Blažková J, Dvořák Z, Blahoš J. Intestinal cell-specific vitamin D receptor (VDR)-mediated transcriptional regulation of CYP3A4 gene. **Biochem Pharmacol 2010** 79(2): 277-287.
- Pavlowski A, Gianfelice A, Pallotto M, Zanchi A, Vara H, Khelfaoui M, Valnegri P, Rezai X, Bassani S, Brambilla D, Kumpošt J, Blahoš J, Roux MJ, Humeau Y, Chelly J, Passafaro M, Giustetto M, Billuart P, Sala C. A postsynaptic signaling pathway that may account for the cognitive defect due to IL1RAPL1 mutation. **Curr Biol 2010** 20(2): 103-115.

- Rulcova A, Prokopova I, Krausova L, Bitman M, Vrzal R, Dvorak Z, Blahos J, Pavek P. Stereoselective interactions of warfarin enantiomers with the pregnane X nuclear receptor in gene regulation of major drug-metabolizing cytochrome P450 enzymes. **J Thromb Haemost 2010** 8(12): 2708-2717.

DRÁBER PAVEL

- Blume Y, Yemets A, Sheremet Y, Nyporko A, Sulimenko V, Sulimenko T, Dráber P. Exposure of β-tubulin regions defined by antibodies on an *Arabidopsis thaliana* microtubule protofilament model and in the cells. **BMC Plant Biol 2010** 10: 29.
- Caracciolo V, D'Agostino L, Dráberová E, Sládková V, Crozier-Fitzgerald C, Agamanolis DP, De Chadarévian JP, Legido A, Giordano A, Dráber P, Katsetos CD. Differential expression and cellular distribution of γ-tubulin and βIII-tubulin in medulloblastomas and human medulloblastoma cell lines. **J Cell Physiol 2010** 223(2): 519-529.
- Dráberová E, Sulimenko V, Sulimenko T, Böhm KJ, Dráber P. Recovery of tubulin functions after freeze-dying in the presence of trehalose. **Anal Biochem 2010** 397(1): 67-72.
- Katsetos CD, Dráberová E, Legido A, Dumontet C, Dráber P. Tubulin targets in the pathobiology and therapy of glioblastoma multiforme. I. class III β-tubulin. **J Cell Physiol 2009** 221(3): 505-513.
- Katsetos CD, Dráberová E, Legido A, Dráber P. Tubulin targets in the pathobiology and therapy of glioblastoma multiforme. II. γ-tubulin. **J Cell Physiol 2009** 221(3): 514-520.
- Kovář J, Ehrlichová M, Šmejkalová B, Zanardi I, Ojima I, Gut I. Comparison of cell death-inducing effect of novel taxane SB-T-1216 and paclitaxel in breast cancer cells. **Anticancer Res 2009** 29(8): 2951-2960.

DRÁBER PETR

- Bugajev V, Bambousková M, Dráberová L, Dráber P. What precedes the initial tyrosine phosphorylation of the high affinity IgE receptor in antigen-activated mast cell? **FEBS Lett 2010** 584(24): 4949-4955.
- Heneberg P, Dráberová L, Bambousková M, Pompach P, Dráber P. Down-regulation of protein-tyrosine phosphatases activates an immune receptor in the absence of its translocation into lipid rafts. **J Biol Chem 2010** 285(17): 12787-12802.
- Shaik GM, Dráberová L, Heneberg P, Dráber P. Vacuolin-1-modulated exocytosis and cell resealing in mast cells. **Cell Signal 2009** 21(8): 1337-1345.
- Tůmová M, Koffer A, Šimíček M, Dráberová L, Dráber P. The transmembrane adaptor protein NTAL signals to mast cell cytoskeleton via the small GTPase Rho. **Eur J Immunol 2010** 40(11): 3235-3245.

DVOŘÁK

- Bříza T, Kejík Z, Králová J, Martásek P, Král V. Synthesis of unsymmetric cyanine dye via merocyanine nad their interaction with DNA. **Coll Czechosl Chem Commun 2009** 78(7-8): 1081-1090.
- Čermák V, Kosla J, Plachý J, Trejbalová K, Hejnar J, Dvořák M. The transcription factor EGR1 regulates metastatic potential of v-*src* transformed sarcoma cells. **Cell Mol Life Sci 2010** 67(20): 3557-3568.
- Kejík Z, Bříza T, Králová J, Martásek P, Král V. Selective recognition of a saccharide-type tumor marker with natural and synthetic ligands: a new trend in cancer diagnosis. **Anal Bioanal Chem 2010** 398(5): 1865-1870.
- Králová J, Kejík Z, Bříza T, Poučková P, Král A, Martásek P, Král V. Porphyrin-cyclodextrin conjugates as a nanosystem for versatile drug delivery and multimodal cancer therapy. **J Med Chem 2010** 53(1): 128-138.
- Kučera A, Pýcha K, Pajer P, Špíšek R, Škába R. Dendritic cell-based immunotherapy induces transient clinical response in advanced rat fibrosarcoma – comparison with preventive anti-tumour vaccination. **Folia Biol (Praha) 2009** 55(4): 119-125.
- Nováková Z, Hubáčková S, Košař M, Janderová-Rossmeislová L, Dobrovolná J, Vašicová P, Vančurová M, Hořejší Z, Hozák P, Bartek J, Hodný Z. Cytokine expression and signaling in drug-induced cellular senescence. **Oncogene 2010** 29(2): 273-284.
- Pajer P, Karafiát V, Pečenka V, Průková D, Dudlová J, Plachý J, Kašparová P, Dvořák M. Industasis, a promotion of tumor formation by nontumorigenic stray cells. **Cancer Res 2009** 69(11): 4605-4612.
- Peková S, Ivánek R, Dvořák M, Rueggeberg S, Leicht S, Li X, Franz T, Kozak T, Vrba J, Koza V, Karas M, Schwarz J, Cetkovský P, Průcha M. Molecular variability of FLT3/ITD mutants and their impact on the differentiation program of 32D cells: Implications for the biological properties of AML blasts. **Leukemia Res 2009** 33(10): 1409-1416.
- Záruba K, Králová J, Řezanka P, Poučková P, Veverková L, Král V. Modified porphyrin-brucine conjugated to gold nanoparticles and their application in photodynamic therapy. **Org Biomol Chem 2010** 8 (14): 3202-3206.
- Zíková M, Corlett A, Bendová Z, Pajer P, Bartůněk P. DISP3, a sterol-sensing domain-containing protein that links thyroid hormone action and cholesterol metabolism. **Mol Endocrinol 2009** 23(4): 520-528.

FILIPP

- Chlubnová I, Filipp D, Spiwok V, Dvořáková H, Daniellou R, Nugier-Chauvin C, Králová B, Ferrières V. Enzymatic synthesis of oligo-D-galactofuranosides and L-arabinofuranosides: from molecular dynamics to immunological assays. **Org Biomol Chem 2010** 8(9): 2092-2102.
- Jirsova K, Neuwirth A, Kalasova S, Vesela V, Merjava S. Mesothelial proteins are expressed in the human cornea. **Exp Eye Res 2010** 91(5): 623-629.

FOREJT

- Mihola O, Trachtulec Z, Vlček Č, Schimenti JC, Forejt J. A mouse speciation gene encodes a meiotic histone H3 methyltransferase. **Science 2009** 323(5912): 373-375.
- Schughart K, Arends D, Andreux P, Balling R, Beyer A, Bezerianos A, Brockmann GA, Crusio WE, Campbell-Tofte J, Denny P, Falcon-Perez JM, Forejt J, Franken P, Hovatta I, Iraqi F, Jansen RC, Kaczmarek L, Kas MJ, Kashofer K, Knapska E, Kolisis F, Kõks S, Lammert F, Möller S, Montagutelli X, Morahan G, Mott R, Pfoertner S, Prins P, Przewlocki R, Ranki A, Santos J, Rihet P, Schalkwyk L, Smit AB, Swertz M, Threadgill D, Vasar E, Zatloukal K. SYSGENET: a meeting report from a new European network for systems genetics. **Mamm Genome 2010** 21(7-8): 331-336.

HEJNAR

- Bakri MM, Sutherland AD, Brown DJ, Veselý P, Crossan C, Scobie L. Assessment of the potential risk of infection associated with *Clostridium difficile* from porcine xenografts. **Xenotransplantation 2009** 16(6): 472-476.
- Blažková J, Trejbalová K, Gondois-Rey F, Halfon P, Philibert P, Guiguen A, Verdin E, Olive D, Van Lint C, Hejnar J, Hirsch I. CpG methylation controls reactivation of HIV from latency. **PLoS Pathogens 2009** 5(8): e1000554.
- Brábek J, Mierke CT, Rösel D, Veselý P, Fabry B. The role of the tissue microenvironment in the regulation of cancer cell motility and invasion. **Cell Commun Signal 2010** 8:22.
- Brühl E, Cihak J, Niedermeier M, Denzel A, Gomez MR, Talke Y, Goebel N, Plachý J, Stangassinger M, Mack M. Important role of IL-3 during initiation of collagen induced arthritis. **Arthritis Rheum 2009** 60(5): 1352-1361.
- Čermák V, Kosla J, Plachý J, Trejbalová K, Hejnar J, Dvořák M. The transcription factor EGR1 regulates metastatic potential of v-*src* transformed sarcoma cells. **Cell Mol Life Sci 2010** 67(20): 3557-3568.
- Hejnar J, Hirsch I. New inhibitors of HDAC to purge latent HIV-1 reservoir. **Epigenomics 2010** 2: 505-506.
- Hirsch I, Hejnar J. Reactivation of HIV-1 latent reservoir by inhibitor of H3K9me2 methyl transferase G9a. **Epigenomics 2010** 2: 506-507.
- Janečková H, Veselý P, Chmelík R. Proving tumour cells by acute nutritional/energy deprivation as a survival threat: a task for microscopy. **Anticancer Res 2009** 29(6): 2339-2345.
- Lukáš J, Mazna P, Valenta T, Doubravská L, Pospíchalová V, Vojtěchová M, Fafílek B, Ivánek R, Plachý J, Novák J, Kořínek V. Dazap2 modulates transcription driven by the Wnt effector TCF-4. **Nucleic Acids Res 2009** 37(9): 3007-3020.
- Mořkovský L, Storchová R, Plachý J, Ivánek R, Divina P, Hejnar J. The Chicken Z chromosome is enriched for genes with preferential expression in ovarian somatic cells. **J Mol Evol 2010** 70: 129-136.
- Mucksová J, Brillard J-P, Hejnar J, Poplštejn M, Kalina J, Bakst M, Yan H, Trefil P. Identification of various testicular cell populations in pubertal and adult cockerels. **Anim Reprod Sci 2009** 114(4): 415-422.
- Pajer P, Karafiát V, Pečenka V, Průková D, Dudlová J, Plachý J, Kašparová P, Dvořák M. Industasis, a promotion of tumor formation by nontumorigenic stray cells. **Cancer Res 2009** 69(11): 4605-4612.
- Pávek P, Pospěchová K, Švecová L, Syrová Z, Stejskalová L, Blažková J, Dvořák Z, Blahoš J. Intestinal cell-specific vitamin D receptor (VDR)-mediated transcriptional regulation of CYP3A4 gene. **Biochem Pharmacol 2010** 79(2): 277-287.
- Plachý J, Kotáb J, Divina P, Reinišová M, Šenigl F, Hejnar J. Proviruses selected for high and stable expression of transduced genes accumulate in broadly transcribed genome areas. **J Virol 2010** 84(9): 4204-4211.
- Svoboda J. Unsung hero Robert C. Gallo. **Science 2009** 9(323): 206-207.
- Trefil P, Bakst MR, Yan H, Hejnar J, Kalina J, Mucksová J. Restoration of spermatogenesis after transplantation of c-Kit positive testicular cells in the fowl. **Theriogenology 2010** 74(9): 1670-1676.
- Tolde O, Rösel D, Mierke CT, Panková D, Folk P, Veselý P, Brábek J. Neoplastic progression of the human breast cancer cell line G3S1 is associated with elevation of cytoskeletal dynamics and upregulation of MT1-MMP. **Int J Oncol 2010** 36(4): 833-839.
- Tolde O, Rösel D, Veselý P, Folk P, Brábek J. The structure of invadopodia in a complex 3D environment. **Eur J Cell Biol 2010** 89(9): 674-680.
- Veselý P, Rösel D, Paňková D, Tolde O, Blase C, Matoušková E, Folk P, Brábek J, Bereiter-Hahn J. Confocal microscopy reveals *Myzitiras* and *Vthela* morphotypes as new signatures of malignancy progression. **Scanning 2009** 31(3): 102-106.

HOLÁŇ

- Furugaki K, Pokorná K, le Pogam C, Aoki M, Reboul M, Bajzik V, Krief P, Janin A, Noguera M-E, West R, Charron D, Chomienne C, Pla M, Moins-Teisserenc H, Padua RA. DNA vaccination with all-*trans* retinoic acid treatment induces long-term survival and elicits specific immune responses requiring $CD4^+$ and $CD8^+$ T-cell activation in an acute promyelocytic leukemia mouse model. **Blood 2010** 115(3): 653-656.
- Holáň V, Pokorná K, Procházková J, Krulová M, Zajícová A. Immunoregulatory properties of mouse limbal stem cells. **J Immunol 2010** 184(4): 2124-2129.
- Kaňková Š, Holáň V, Zajícová A, Kodym P, Flegr J. Modulation of immunity in mice with latent toxoplasmosis – an experimental support for the immunosuppression hypothesis of Toxoplasma-induced changes in reproduction of mice and humans. **Parasitol Res 2010** (6): 1421-1427.
- Procházková J, Frič J, Pokorná K, Neuwirth A, Krulová M, Zajícová A, Holáň V. Distinct regulatory roles of transforming growth factor-β and interleukin-4 in the development and maintenance of natural and induced $CD4^+$ $CD25^+$ $Foxp3^+$ regulatory T cells. **Immunology 2009** 128(1 Suppl): e670-e678.

HOŘEJŠÍ

- Hořejší V, Otáhal P, Brdička T. LAT – an important raft-associated transmembrane adaptor protein. **FEBS J 2010** 277(21): 4383-4397.
- Otáhal P, Angelisová P, Hrdinka M, Brdička T, Novák P, Drbal K, Hořejší V. A new type of membrane raft-like microdomains and their possible involvement in TCR signaling. **J Immunol 2010** 184(7): 3689-3696.
- Šmídková M, Müller M, Thönes N, Piuko K, Angelisová P, Velemínský J, Angelis K. Transient expression of human papillomavirus type 16 virus-like particles in tobacco and tomato using a tobacco rattle virus expression vector. **Biol Plantarum 2010** 54(3): 451-460.
- Wu W, Slaastad HS, De la Rosa Carrillo D, Frey T, Tjonnfjord GE, Boretti E, Aasheim HC, Hořejší V, Lund-Jaohnsen F. Antibody array analysis with label-based detection and resolution of protein size. **Mol Cell Proteomics 2009** 8(2): 245-257.

HOZÁK

- Castano E, Philimonenko V, Kahle M, Fukalová J, Kalendová A, Yildrim S, Dzijak R, Dingová H, Hozák P. Actin complexes in the cell nucleus: new stones in an old field. **Histochem Cell Biol 2010** 133(6): 607-626.
- Dingová H, Fukalová J, Maninová M, Philimonenko V, Hozák P. Ultrastructural localization of actin and actin-binding proteins in the nucleus. **Histochem Cell Biol. 2009** 131(3): 425-434.
- Nováková Z, Hubáčková S, Košař M, Janderová-Rossmeislová L, Dobrovolná J, Vašicová P, Vančurová M, Hořejší Z, Hozák P, Bartek J, Hodný Z. Cytokine expression and signaling in drug-induced cellular senescence. **Oncogene 2010** 29(2): 273-284.
- Philimonenko VV, Janáček J, Harata M, Hozák P. Transcription-dependent rearrangements of actin and nuclear myosin I in the nucleolus. **Histochem Cell Biol 2010** 134(3): 243-249.
- Sobol M, Philimonenko VV, Hozák P. Comparison of methods of high-pressure freezing and automated freeze-substitution of suspension cells combined with LR White embedding.
- **Histochem Cell Biol 2010** 134(6): 631-641.

JANŠČÁK

- Dietschy T, Shevelev I, Pena-Diaz J, Hühn D, Kuenzle S, Mak R, Miah MF, Hess D, Fey M, Hottiger MO, Janscak P, Stagljar I. p300-mediated acetylation of the Rothmund-Thomson-syndrome gene product RECQL4 regulates its subcellular localization. **J Cell Sci 2009** 122(Pt 8): 1258-1267.
- Kanagaraj R, Huehn D, Mackellar A, Menigatti M, Zheng L, Urban V, Shevelev I, Greenleaf AL, Janscak P. RECQ5 helicase associates with the C-terminal repeat domain of RNA polymerase II during productive elongation phase of transcription. **Nucleic Acids Res 2010** 38(22): 8131-8140.
- Schwendener S, Raynard S, Paliwal S, Cheng A, Kanagaraj R, Shevelev I, Stark JM, Sung P, Janscak P. Physical interaction of RECQ5 helicase with RAD51 facilitates its anti-recombinase activity. **J Biol Chem 2010** 285(21): 15739-15745.
- Yodh JG, Stevens BC, Kanagaraj R, Janscak P, Ha T. BLM helicase measures DNA unwound before switching strands and hRPA promotes unwinding reinitiation. **EMBO J 2009** 28(4): 405-416.
- Zheng L, Kanagaraj R, Mihaljevic B, Schwendener S, Sartori AA, Gerrits B, Shevelev I, Janscak P. MRE11 complex links RECQ5 helicase to sites of DNA damage. **Nucleic Acids Res 2009** 37(8): 2645-2657.

KOŘÍNEK

- Fujimura N, Taketo MM, Mori M, Kořínek V, Kozmik Z. Spatial and temporal regulation of Wnt/β-catenin signaling is essential for development of the retinal pigment epithelium. **Dev Biol 2009** 334(1): 31-45.
- Klíma M, Zájedová J, Doubravská L, Anděra L. Functional analysis of the posttranslational modifications of the death receptor 6. **Biochim Biophys Acta-Mol Cell Res 2009** 1793(10): 1579-1587.
- Lukáš J, Mazna P, Valenta T, Doubravská L, Pospíchalová V, Vojtěchová M, Fafílek B, Ivánek R, Plachý J, Novák J, Kořínek V. Dazap2 modulates transcription driven by the Wnt effector TCF-4. **Nucleic Acids Res 2009** 37(9): 3007-3020.
- Turečková J, Vojtěchová M, Krausová M, Šloncová E, Kořínek V. Focal Adhesion Kinase functions as an Akt downstream target in migration of colorectal cancer cells. **Transl Oncol 2009** 2(4): 281-290.

KOZMIK

- Fujimura N, Taketo MM, Mori M, Kořínek V, Kozmik Z. Spatial and temporal regulation of Wnt/β-catenin signaling is essential for development of the retinal pigment epithelium. **Dev Biol 2009** 334(1): 31-45.
- Machoň O, Krešlová J, Růžičková J, Vacík T, Klímová L, Fujimura N, Láchová J, Kozmik Z. Lens morphogenesis is dependent on Pax6-mediated inhibition of the canonical Wnt/β-catenin signaling in the lens surface ectoderm. **Genesis 2010** 48(2): 86-95.
- Růžičková J, Piatigorsky J, Kozmik Z. Eye-specific expression of an ancestral jellyfish *PaxB* gene interferes with *Pax6* function despite its conserved Pax6/Pax2 characteristics. **Int J Dev Biol 2009** 53(4): 469-482.
- Vopálenský P, Kozmik Z. Eye evolution: common use and independent recruitment of genetic components. **Phil Trans Roy Soc B Biol Sci 2009** 364(1531): 2819-2832.
- Vopálenský P, Růžičková J, Pavlů B, Kozmik Z. A lens-specific co-injection marker for medaka transgenesis. **Biotechniques 2010** 48(3): 235-236.

LIPOLDOVÁ

- Drahota J, Lipoldová M, Volf P, Rohoušová I. Specificity of anti-saliva immune response in mice repeatedly bitten by *Phlebotomus sergenti*. **Parasite Immunol 2009** 31(12): 766-770.
- Gusareva E, Bragina EJ, Buinova SN, Chernyak BA, Puzyrev VP, Ogorodova LM, Lipoldová M. Chromosome 12q24.3 controls sensitization to cat allergen in patients with asthma from Siberia, Russia. **Immunol Lett 2009** 125(1): 1-6.
- Gusareva E, Havelková H, Blažková H, Kosařová M, Kučera P, Král V, Salyakina D, Mulller-Myhsok B, Lipoldová M. Mouse to human comparative genetics reveals a novel immunoglobulin E-controlling locus on Hsa8q12. **Immunogenetics 2009** 61(1): 15-25.
- Kobets T, Badalová J, Grekov I, Havelková H, Svobodová M, Lipoldová M. *Leishmania* parasite detection and quantification using PCR-ELISA. **Nat Protoc 2010** 5(6): 1074-1080.
- Kurey I, Kobets T, Havelková H, Slapničková M, Quan L, Trtková K, Grekov I, Svobodová M, Stassen APM, Hutson A, Demant P, Lipoldová M. Distinct genetic control of parasite elimination, dissemination, and disease after *Leishmania major* infection. **Immunogenetics 2009** 61(9): 619-633.
- Lipoldová M, Havelková H, Badalová J, Vojtíšková J, Quan L, Krulová M, Sohrabi Y, Stassen APM, Demant P. Loci controlling lymphocyte production of interferon γ after alloantigen stimulation *in vitro* and their co-localization with genes controlling lymphocyte infiltration of tumors and tumor susceptibility. **Cancer Immunol Immunother 2010** 59(2): 203-213.

REINIŠ

- Bubeník J. Genetically modified vaccines augment the efficacy of cancer surgery and chemotherapy. **Folia Biol (Praha) 2009** 55(6): 199-200.
- Bubeník J, Šímová J. Genetically modified cellular vaccines against human papillomavirus type 16 (HPV16)-associated tumors: adjuvant treatment of minimal residual disease after surgery/chemotherapy. **J BUON 2009** 14(1): 169-173.
- Indrová M, Bieblová J, Rossowska J, Kuropka P, Pajtasz-Piasecka E, Bubeník J, Reiniš M. HPV 16-associated tumours: IL-12 can repair the absence of cytotoxic and proliferative responses of tumour infiltrating cells after chemotherapy. **Int J Oncol 2009** 34(1): 173-180.
- Kemal KS, Reiniš M, Weiser B, Burger H. Methods for viral RNA isolation and PCR amplification for sequencing of near full-length HIV-1 genomes. **Methods Mol Biol 2009** 485: 3-14.
- Linka M, Brůčková M, Malý M, Vandasová J, Stanková M, Reiniš M. A study of HIV-1 genetic diversity in the Czech Republic: 1986-2007. **Cent Eur J Publ Health 2009** 16(4): 175-177.
- Nussbaumerová M, Srp J, Máša M, Hradílek M, Šanda M, Reiniš M, Horn M, Mareš M. Single- and double-headed chemical probes for detection of active cathepsin D in a cancer cell proteome. **Chembiochem 2010** 11(11): 1538-1541.
- Pajtasz-Piasecka E, Indrová M. Dendritic cell-based vaccines for the therapy of experimental tumors. **Immunotherapy 2010** 2(2): 257-268.
- Reiniš M. Immunotherapy of MHC class I-deficient tumors. **Future Oncol 2010** 6(10): 1577-1589.

- Reinis M, Stepanek I, Simova J, Bieblova J, Pribylova H, Indrova M, Bubenik J. Induction of protective immunity against MHC class I-deficient, HPV16-associated tumours with peptide and dendritic cell-based vaccines. **Int J Oncol 2010** 36(3): 545-551.
- Šímová J, Indrová M, Bieblová J, Mikyšková R, Bubeník J, Reiniš M. Therapy for minimal residual tumor disease: β-galactosylceramide inhibits the growth of recurrent HPV16-associated neoplasms after surgery and chemotherapy. **Int J Cancer 2010** 126(12): 2997-3004.

ŘEZÁČOVÁ

- Boura E, Rezabkova L, Brynda J, Obsilova V, Obsil T. Structure of the human FOXO4-DBD-DNA complex at 1.9 Å resolution reveals new details of FOXO binding to the DNA. **Acta Crystallogr D 2010** 66 (Pt 12): 1351-1357.
- Dostál J, Brynda J, Hrušková O, Sieglová I, Pichová I, Řezáčová P. The crystal structure of the secreted aspartic protease 1 from *Candida parapsilosis* in complex with pepstatin A. **J. Struct Biol 2009** 167(2): 145-152.
- Gitto R, Agnello S, Ferro S, De Luca L, Vullo D, Brynda J, Mader P, Supuran CT, Chimirri A. Identification of 3,4-dihydroisoquinoline-2(1H)-sulfonamides as potent carbonic anhydrase inhibitors: synthesis, biological evaluation, and enzyme-ligand X-ray studies. **J Med Chem 2010** 53(6): 2401-2408.
- Kohoutová J, Kutá Smatanová I, Brynda J, Lapkouski M, Revuelta JL, Arellano JB, Ettrich R. Crystallization and preliminary crystallographic characterization of the extrinsic PsbP protein of photosystem II from *Spinacia oleracea*. **Acta Crystallogr Sect F 2009** 65(Pt 2): 111-115.
- Kovářová Z, Chmelař J, Sanda M, Brynda J, Mareš M, Rezáčová P. Crystallization and diffraction analysis of the serpin IRS-2 from the hard tick *Ixodes ricinus*. **Acta Crystallogr Sect F 2010** 66 (Pt 11): 1453-1457.
- Prudnikova T, Gavira JA, Rezacova P, Pineda Molina E, Hunalove I, Sviridova E, Shmidt V, Kohoutova J, Kuty M, Kaftan D, Vacha F, Garcia-Ruiz JM, Kuta-Smatanova I. Toward the crystallization of photosystem II core complex from *Pisum sativum* L. **Cryst Growth Des 2010** 10(8): 3391-3396.
- Prudnikova T, Mozga T, Řezáčová P, Chaloupková R, Sato Y, Nagata Y, Brynda J, Kutý M, Damborský J, Kutá-Smatanová I. Crystallization and preliminary X-ray analysis of a novel haloalkane dehalogenase DbeA from *Bradyrhizobium elkani* USDA94. **Acta Crystallogr Sect F 2009** 65(Pt 4): 353-356.
- Řezáčová P, Pokorná J, Brynda J, Kožíšek M, Cígler P, Lepšík M, Fanfrlík J, Řezáč T, Grantz Šašková K, Sieglová I, Plešek J, Šícha V, Grüner B, Oberwinkler H, Sedláček J, Kräusslich HG, Hobza P, Král V, Konvalinka J. Design of HIV protease inhibitors based on inorganic polyhedral metallacarboranes. **J Med Chem 2009** 52(22): 7132-7141.
- Salát J, Paesen GC, Řezáčová P, Kotsyfakis M, Kovářová Z, Šanda M, Majtán J, Grunclová L, Horká H, Andersen JF, Brynda J, Horn M, Nunn MA, Kopáček P, Kopecký J, Mareš M. Crystal structure and functional characterization of an immunomodulatory salivary cystatin from the soft tick *Ornithodoros moubata*. **Biochem J 2010** 429(1): 103-112.
- Sasková KG, Kozísek M, Rezácová P, Brynda J, Yashina T, Kagan RM, Konvalinka J. Molecular characterization of clinical isolates of human immunodeficiency virus resistant to the protease inhibitor darunavir. **J Virol 2009** 83(17): 8810-8848.
- Skultety L, Jankovičová B, Svobodová Z, Mader P, Řezáčová P, Dubrovčáková M, Lakota J, Bílková Z. Identification of carbonic anhydrase I immunodominant epitopes recognized by specific autoantibodies which indicate an improved prognosis in patients with malignancy after autologous stem cell transplantation. **J Proteome Res 2010** 9(10): 5171-5179.
- Sviridova E, Bumba L, Rezacova P, Prochazkova K, Kavan D, Bezouska K, Kuty M, Sebo P, Kuta Smatanova I. Crystallization and preliminary crystallographic characterization of the iron-regulated outer membrane lipoprotein FrpD from *Neisseria meningitidis*. **Acta Crystallogr Sect F 2010** 66(Pt 9): 1119-1123.
- Wolfova J, Smatanova IK, Brynda J, Mesters JR, Lapkouski M, Kuty M, Natalello A, Chatterjee N, Chern SY, Ebbel E, Ricci A, Grandori R, Ettrich R, Carey J. Structural organization of WrbA in apo- and holoprotein crystals. **Biochim Biophys Acta 2009** 1794(9): 1288-1298.

SEDLÁČEK

- Lettau I, Hattermann K, Held-Feindt J, Brauer R, Sedlacek R, Mentlein R. Matrix metalloproteinase-19 (MMP19) is highly expressed in astroglial tumors and promotes invasion of glioma cells. **J Neuropathol Exp Neurol 2010** 69(3): 215-223.
- Lokossou AA, Rietman H, Wang M, Krenek P, van der Schoot H, Henken B, Hoekstra R, Vleeshouwers VGGA, van der Vossen EAG, Visser RGF, Jacobsen E, Vosman B. Diversity, distribution, and evolution of *Solanum bulbocastanum* late blight resistance genes. **Mol Plant Microbe Interact 2010** 23(9): 1206-1216.
- Muller M, Beck I, Gadesmann J, Karschuk N, Paschen A, Proksch E, Djonov V, Reiss K, Sedlacek R. MMP19 is upregulated during melanoma progression and increases invasion of melanoma cells. **Mod Pathol 2010** 23(4): 511-521.
- Sarnova L, Malik R, Sedlacek R, Svoboda P. Shortcomings of short hairpin RNA-based transgenic RNA interference in mouse oocytes. **J Negat Results Biomed 2010** 9: 8.

STANĚK

- Huranová M, Hnilicová J, Fleischer B, Cvačková Z, Staněk D. A mutation linked to retinitis pigmentosa in HPRP31 causes protein instability and impairs its interactions with spliceosomal snRNPs. **Hum Mol Genet 2009** 18(11): 2014-2023.
- Huranová M, Ivani I, Benda A, Poser I, Brody Y, Hof M, Shav-Tal Y, Neugebauer KM, Staněk D. The differential interaction of snRNPs with pre-mRNA reveals splicing kinetics in living cells. **J Cell Biol 2010** 191: 75-86.
- Huranová M, Jablonski JA, Benda A, Hof M, Staněk D, Caputi M. *In vivo* detection of RNA-binding protein interactions with cognate RNA sequences by fluorescence resonance energy transfer. **RNA 2009** 15(11): 2063-2071.

SVOBODA

- Flemr M, Ma J, Schultz RM, Svoboda P. P-body loss is concomitant with formation of a messenger RNA storage domain in mouse oocytes. **Biol Reprod 2010** 82(5): 1008-1017.
- Ma J, Flemr M, Stein P, Berninger P, Malík R, Zavolan M, Svoboda P, Schultz RM. MicroRNA activity is suppressed in mouse oocytes. **Curr Biol 2010** 20(3): 265-270.

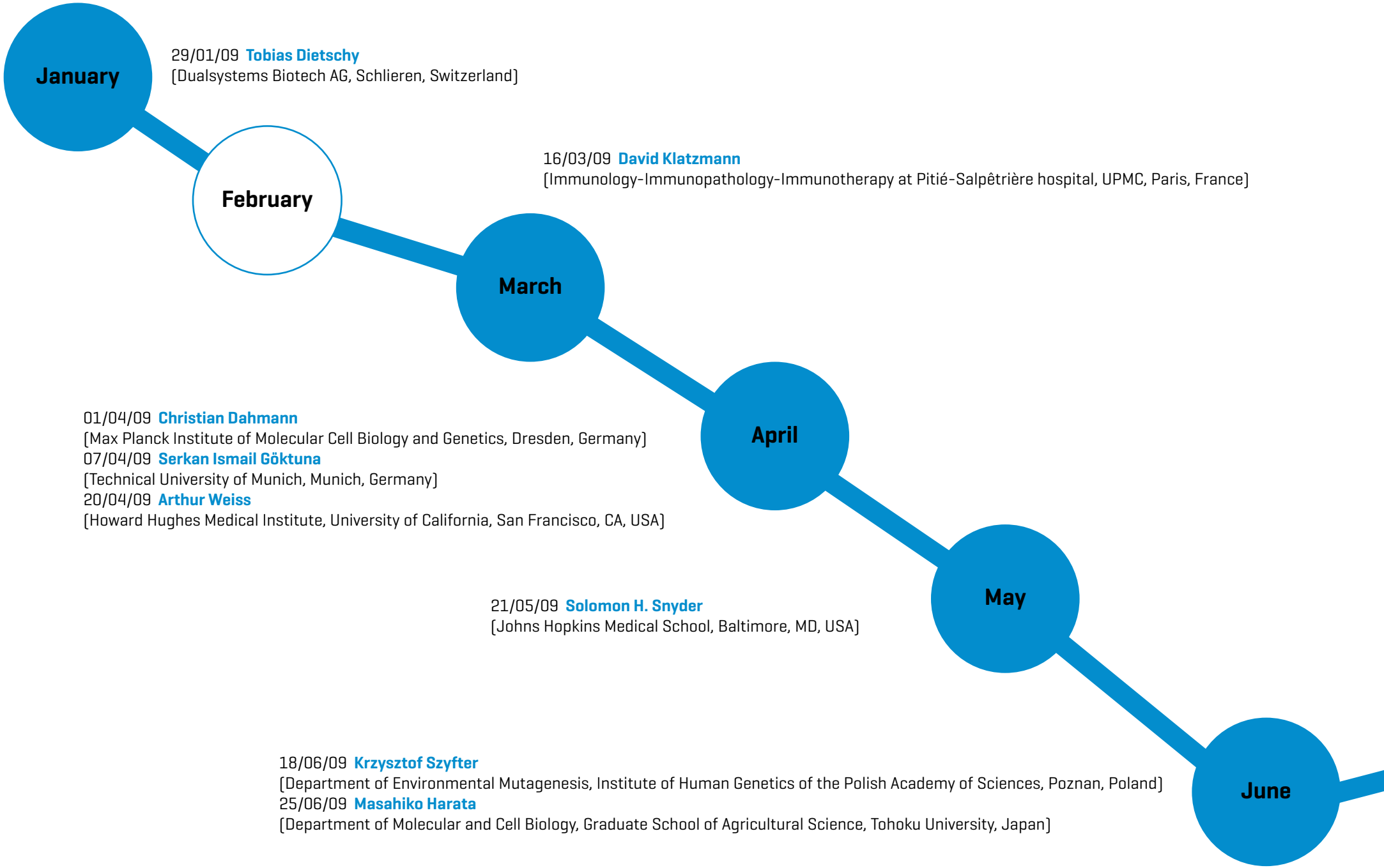
- Sarnova L, Malik R, Sedlacek R, Svoboda P. Shortcomings of short hairpin RNA-based transgenic RNA interference in mouse oocytes. **J Negat Results Biomed 2010** 9: 8.
- Svoboda P, Flemr M. The role of miRNAs and endogenous siRNAs in maternal-to-zygotic reprogramming and the establishment of pluripotency. **EMBO Rep 2010** 11(8): 590-597.
- Svoboda P. Journal club. A molecular biologist explores how new genomic tools can be applied to wild animals. **Nature 2010** 465(7298): 529.
- Svoboda P. Why mouse oocytes and early embryos ignore miRNAs? **RNA Biol 2010** 7(5): 54-58.

VLČEK

- Mercer RG, Callister SJ, Lipton MS, Pasa-Tolic L, Strnad H, Paces V, Beatty JT, Lang AS. Loss of the response regulator CtrA causes pleiotropic effects on gene expression but does not affect growth phase regulation in *Rhodobacter capsulatus*. **J Bacteriol 2010** 192(11): 2701-2710.
- Mihola O, Trachtulec Z, Vlček Č, Schimenti JC, Forejt J. A mouse speciation gene encodes a meiotic histone H3 methyltransferase. **Science 2009** 323(5912): 373-375.
- Strnad H, Lacina L, Kolář M, Čada Z, Vlček Č, Dvořánková B, Betka J, Plzák J, Chovanec M, Šáchová J, Valach J, Urbanová M, Smetana K Jr. Head and neck squamous cancer stromal fibroblasts produce growth factors influencing phenotype of normal human keratinocytes. **Histochem Cell Biol 2010** 133(2): 201-211.
- Strnad H, Lapidus A, Pačes J, Ulbrich P, Vlček Č, Pačes V, Haselkorn R. Complete genome sequence of the photosynthetic purple nonsulfur bacterium *Rhodobacter capsulatus* SB 1003. **J Bacteriol 2010** 192(13): 3545-3546.
- Uhlík O, Ječná K, Macková M, Vlček Č, Hroudová M, Demnerová K, Pačes V, Macek T. Biphenyl-metabolizing bacteria in the rhizosphere of horseradish and bulk soil contaminated by polychlorinated biphenyls as revealed by stable isotope probing. **Appl Environ Microbiol 2009** 75(20): 6471-6477.
- Urbanová M, Plzák J, Strnad H, Betka J. Circulating nucleic acids as a new diagnostic tool. **Cell Mol Biol Lett 2010** 15(2): 242-259.
- Záveská Drábková L, Kirschner J, Štěpánek J, Záveský L, Vlček Č. Analysis of nrDNA polymorphism in closely related diploid sexual, tetraploid sexual and polyploid agamospermous species. **Plant System Evol 2009** 278(1-2): 85, app. 1-11.
- Záveská Drábková L, Vlček Č. DNA variation within Juncaceae: Comparison of impact of organelle regions on phylogeny. **Plant System Evol 2009** 278(3): 169-186.
- Záveská Drábková L, Vlček Č. Molecular phylogeny of the genus *Luzula* DC. (Juncaceae, Monocotyledones) based on plastome and nuclear ribosomal regions: A case of incongruence, incomplete lineage sorting and hybridisation. **Mol Phylogenet Evol 2010** 57(2): 536-551.

OTHER PUBLICATIONS UNDER IMG AFFILIATION (from former groups or core facilities)

- Divina P, Kvitkovicova A, Buratti E, Vorechovsky I. *Ab initio* prediction of mutation-induced cryptic splice-site activation and exon skipping. **Eur J Hum Genet 2009** 17(6): 759-765.
- Ferjentsik Z, Sindelka R, Jonak J. Expression patterns of Src-family tyrosine kinases during *Xenopus laevis* development. **Int J Dev Biol 2009** 53(1): 163-168.
- Lukáš J, Mazna P, Valenta T, Doubravská L, Pospíchalová V, Vojtěchová M, Fafílek B, Ivánek R, Plachý J, Novák J, Kořínek V. Dazap2 modulates transcription driven by the Wnt effector TCF-4. **Nucleic Acids Res 2009** 37(9): 3007-3020.
- Mořkovský L, Storchová R, Plachý J, Ivánek R, Divina P, Hejnar J. The Chicken Z chromosome is enriched for genes with preferential expression in ovarian somatic cells. **J Mol Evol 2010** 70: 129-136.
- Motácková V, Sanderová H, Zídek L, Novácek J, Padrta P, Svenková A, Korelusová J, Jonák J, Krásný L, Sklenár V. Solution structure of the N-terminal domain of *Bacillus subtilis* delta subunit of RNA polymerase and its classification based on structural homologs. **Proteins 2010** 78(7): 1807-1810.
- Peková S, Ivánek R, Dvořák M, Rueggeberg S, Leicht S, Li X, Franz T, Kozak T, Vrba J, Koza V, Karas M, Schwarz J, Cetkovský P, Průcha M. Molecular variability of FLT3/ITD mutants and their impact on the differentiation program of 32D cells: Implications for the biological properties of AML blasts. **Leukemia Res 2009** 33(10): 1409-1416.
- Plachý J, Kotáb J, Divina P, Reinišová M, Šenigl F, Hejnar J. Proviruses selected for high and stable expression of transduced genes accumulate in broadly transcribed genome areas. **J Virol 2010** 84(9): 4204-4211.
- Pšenička M, Vancová M, Koubek P, Těšitel J, Linhart O. Fine structure and morphology of sterlet (*Acipenser ruthenus* L. 1758) spermatozoa and acrosin localization. **Anim Reprod Sci 2009** 111(1): 3-16.
- Šanderová H, Tišerová H, Barvík I, Sojka L, Jonák J, Krásný L. The N-terminal region is crucial for the thermostability of the G-domain of *Bacillus stearothermophilus* EF-Tu. **Biochim Biophys Acta 2010** 1804(1): 147-155.
- Tomasetti M, Amati M, Santarelli L, Alleva R, Neužil J. Malignant mesothelioma: biology, diagnosis and therapeutic approaches. **Curr Mol Pharmacol 2009** 2(2): 1


January
29/01/09 Tobias Dietschy
[Dualsystems Biotech AG, Schlieren, Switzerland]
February
16/03/09 David Klatzmann
[Immunology-Immunopathology-Immunotherapy at Pitié-Salpêtrière hospital, UPMC, Paris, France]
March
01/04/09 Christian Dahmann
[Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany]
07/04/09 Serkan Ismail Göktuna
[Technical University of Munich, Munich, Germany]
20/04/09 Arthur Weiss
[Howard Hughes Medical Institute, University of California, San Francisco, CA, USA]
April
21/05/09 Solomon H. Snyder
[Johns Hopkins Medical School, Baltimore, MD, USA]
May
18/06/09 Krzysztof Szyfter
[Department of Environmental Mutagenesis, Institute of Human Genetics of the Polish Academy of Sciences, Poznan, Poland]
25/06/09 Masahiko Harata
[Department of Molecular and Cell Biology, Graduate School of Agricultural Science, Tohoku University, Japan]
June

09/11/09 **Jeffrey Good**
[Department of Evolutionary Genetics, Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany]

06/10/09 **Olivier Cuvillier**
[Sphingolipids & Cancer Research Laboratory, INSERM, Toulouse, France]
21/10/09 **Jonathan C. Howard**
[Institute for Genetics, University of Cologne, Cologne, Germany]
26/10/09 **David Margolis**
[Michael Hooker Research Center, University of North Carolina at Chapel Hill, NC, USA]

01/09/09 **Harry F. Noller**
[Center for Molecular Biology of RNA, University of California, Santa Cruz, CA, USA]
22/09/09 **Vladimír Varga**
[Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany]

Seminar Speakers 2009

Seminar Speakers 2010

January

February

03/02/10 **Cheng Zhu**
[Institute for Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, GA, USA]
12/02/10 **Shazib Pervaiz**
[National University of Singapore, Singapore]

March

03/03/10 **Wolfgang Zachariae**
[Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany]

April

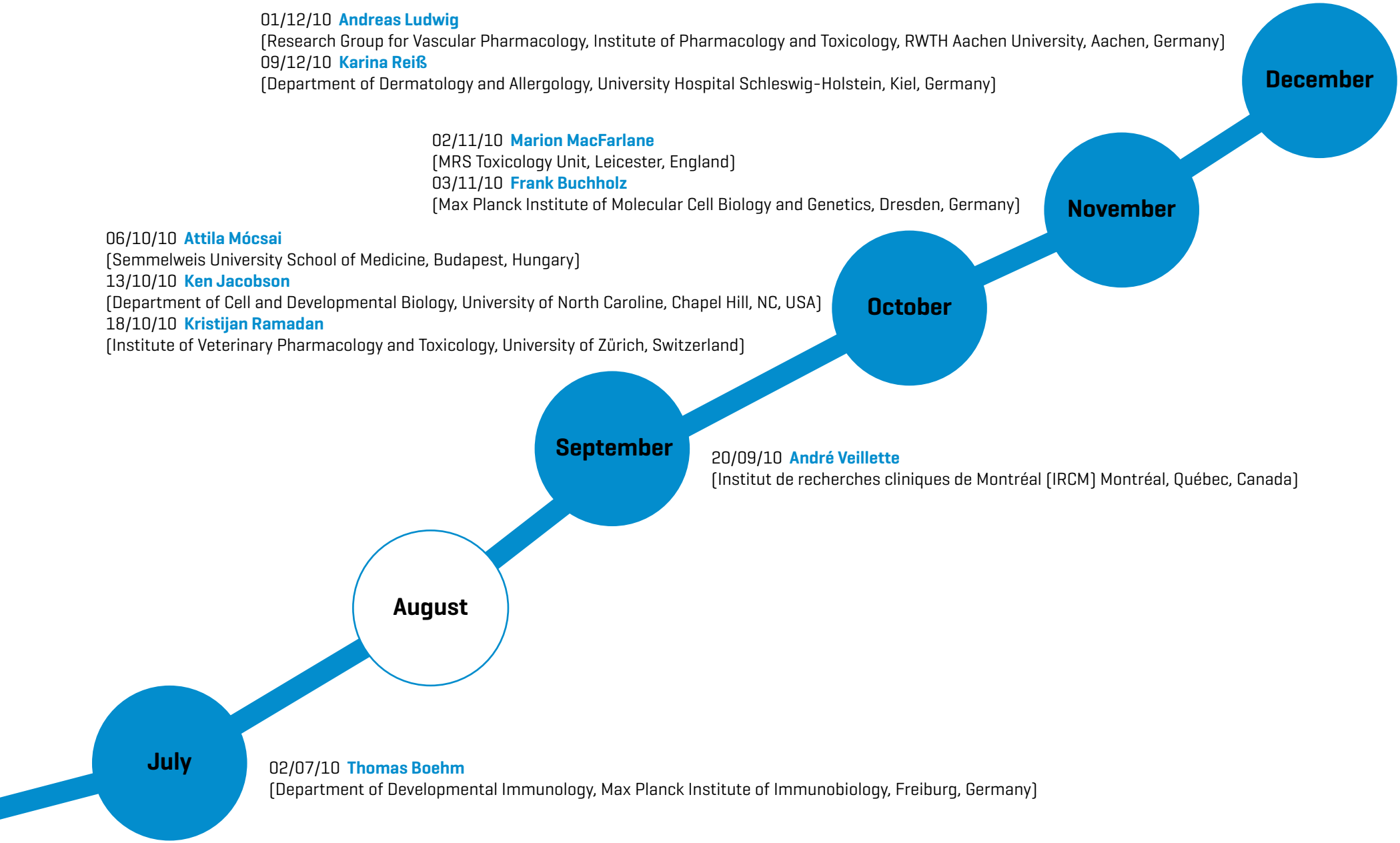
07/04/10 **Kanagaraj Radhakrishnan**
[Institute of Molecular Cancer Research, University of Zurich, Switzerland]
28/04/10 **Petko Petkov**
[The Jackson Laboratory, Bar Harbor, ME, USA]

May

26/05/10 **Jonathon Howard**
[Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany]

June

11/06/10 **Anatoly Ruvinsky**
[Institute for Genetics and Bioinformatics, University of New England, Armidale, Australia]



01/12/10 **Andreas Ludwig**
[Research Group for Vascular Pharmacology, Institute of Pharmacology and Toxicology, RWTH Aachen University, Aachen, Germany]
09/12/10 **Karina Reiß**
[Department of Dermatology and Allergology, University Hospital Schleswig-Holstein, Kiel, Germany]

02/11/10 **Marion MacFarlane**
[MRS Toxicology Unit, Leicester, England]
03/11/10 **Frank Buchholz**
[Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany]

06/10/10 **Attila Mócsai**
[Semmelweis University School of Medicine, Budapest, Hungary]
13/10/10 **Ken Jacobson**
[Department of Cell and Developmental Biology, University of North Caroline, Chapel Hill, NC, USA]
18/10/10 **Kristijan Ramadan**
[Institute of Veterinary Pharmacology and Toxicology, University of Zürich, Switzerland]

20/09/10 **André Veillette**
[Institut de recherches cliniques de Montréal (IRCM) Montréal, Québec, Canada]

02/07/10 **Thomas Boehm**
[Department of Developmental Immunology, Max Planck Institute of Immunobiology, Freiburg, Germany]

Highlights of 2009-2010

Organization & Development

Development of the Institute complex further advanced

Construction of the kindergarten with gym and of the new guest house was completed within 2010, and by the end of the year both facilities were ready for opening. Pavilion CH underwent a thorough reconstruction and is now housing the non-mouse (mainly chicken) experimental animal facility.






New instruments

In 2010 we started operation of a new histology unit equipped with Tissue processor Leica ASP200S, Paraffin embedding station Leica EG1150H and Microtome RM2255 (operated by the group of Vladimír Kořínek). In pavilion CH a new orthovoltage X-ray instrument T-200 (Wolf-Medizintechnik) for regulated radiation of cells and mice was installed. The mouse animal facility of the Institute was equipped with new individually ventilated breeding cages.

New division

A new division of IMG was established in 2009 with the aim to support preparation and organization of project **BIOCEV (Biotechnology and Biomedicine Centre of The Academy of Sciences and Charles University) in Vestec near Prague,** a joint project of six institutes of the Academy of Sciences of the Czech Republic (Institute of Molecular Genetics, Institute of Biotechnology, Institute of Microbiology, Institute of Physiology, Institute of Experimental Medicine, and Institute of Macromolecular Chemistry) and two faculties of Charles University in Prague (Faculty of Science and First Faculty of Medicine), whose application was completed and sent to the European Commission for approval at the end of 2010. Further information can be found at **http://www.biocev.eu/**.

Awards & Honours

2009

Jiří Forejt – Prize of the Minister of Education, Youth and Sports of the Czech Republic for outstanding results in research, experimental development and innovations for 2009
Laboratory of Jiří Forejt (Zdeněk Trachtulec, Soňa Gregorová, Petr Jansa, David Homolka, Ondřej Mihola) – Prize of the Academy of Sciences of the Czech Republic
Ondřej Ballek – Prize for the best diploma thesis awarded by the Dean of Charles University, Prague
Daniel Smrž (and the Laboratory of Petr Dráber) – Prize of the Czech Immunological Society for the best paper by a young immunologist in 2008
Petr Heneberg – Bolzano Award awarded by the Rector of Charles University, Prague
Václav Pačes – Prize of the Economia publishing house, Economia a.s.
Václav Hořejší – The Sir Hans Krebs Lecture and Medal awarded by FEBS
Daniel Smrž – Award of the Czech Society for Analytical Cytology
Jiří Bartek – The Shay Shacknai Prize for Cancer Research, Hebrew University of Jerusalem; Medal of the Faculty of Medicine, Charles University

2010

Ondřej Mihola – Arnold Beckman Prize for the best publication in the field of genetics, Beckman Coulter CR and the Czech Society for Biochemistry and Molecular Biology
Václav Pačes – Medal of Emil Votoček awarded by the Institute of Chemical Technology, Prague
Ondřej Mihola – Scopus Award for year 2009 awarded by the prominent scientific publisher Elsevier BV
Martina Huranová – Josef Hlávka Award for the best students and young talented researchers by the Hlávka Foundation
Jan Svoboda – National Prize of the Czech Government "Česká hlava" (Czech Brains)
Jiří Bartek – Neuron 2010 for life-long merits in medicine awarded by Karel Janeček Foundation

Highlights of 2009-2010

Seminars & Conferences Organized/Co-organized by IMG

Most of these events were held in the new conference hall of IMG named after the founder of the Institute "Milan Hašek Auditorium".

Conferences:

2009

05/06	2nd IMG PhD Conference
01-03/10	**EMBO Workshop on Mitochondria, Apoptosis and Cancer** (keynote lecture by Guido Kroemer, INSERM, Villejuif, France)
11/12	Annual IMG Conference

2010

25-26/03	T-cell Activation & Technologies
29/04-05/05	**Centennial Retrovirus Meeting** (keynote lecture by David Baltimore, California Institute of Technology, Pasadena, USA)
20-21/05	**ECBS 2010 Prague** – The Second European Chemical Biology Symposium (keynote lecture by James Inglese, NIH, Bethesda, USA)
04/06	3rd IMG PhD Conference
08-10/06	**EMBO Young Scientists Forum**
01-04/09	**52nd Symposium of the Society for Histochemistry** (The Robert Feulgen lecture by Stefan W. Hell, MPI, Göttingen, Germany)
17/12	Annual IMG Conference

Regular weekly Institute seminars – speakers:

2009

Pavel Vopálenský, Stanislav Vinopal, Matyáš Flemr, Miluše Hroudová, Jan Kosla, Jan Konvalinka, Radislav Sedláček, Vlastimil Král, Adéla Broučková, Soňa Hubáčková, Petr Bartůněk, Jan Tachezy, Christian Dahmann, Julius Lukeš, Stanislav Kmoch, Kateřina Trejbalová, Jana Procházková, Marek Jindra, Václav Urban, Ivan Novotný, Petr Šimeček, Vladimír Varga, Milan Reiniš, Tetyana Kobets, Jolana Turečková, Jonathan C. Howard, Magda Tůmová, Jaroslav Blahoš, Vladimíra Šourková, Peter Dráber, Leoš Valášek, Libor Krásný

2010

Zbyněk Kozmik, Vadym Sulimenko, Jana Nejepínská, Markéta Urbanová, Cheng Zhu, Irena Moserová, Rena Brauer, Wolfgang Zachariae, Libor Macůrek, Jana Blažková, Kanagaraj Radhakrishnan, Jana Procházková, Martina Huranová, Petko Petkov, Sukriye Yildirim, Pavel Mader, Jana Oujezdská, Jonathon Howard, Tanmoy Bhattacharyya, David Sedlák, André Veillette, Igor Grekov, Attila Mócsai, Ken Jacobson, Bohumil Fafílek, Jiří Kumpošt, Frank Buchholz, Martina Kovářová, Ladislav Anděra, Andreas Ludwig

PhD Programme

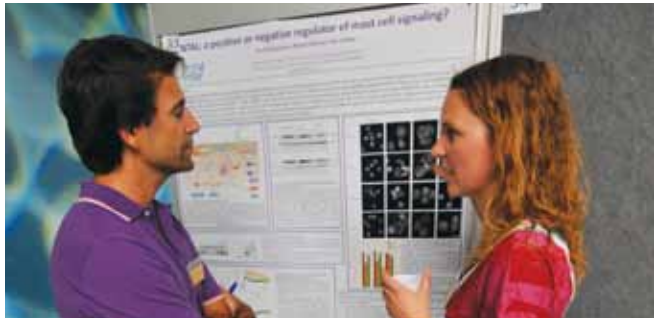
A significant part of our scientific community is represented by about 100 PhD students (20 % international from both EU and non-EU countries), who significantly influence the atmosphere at the Institute and strongly contribute to its scientific output. Therefore, one of our priorities is to offer an appealing PhD programme that will attract the best students and will provide them with high-quality training for a career in molecular, cell and developmental biology, immunology, genetics, and virology. The PhD programme and related topics are organized by the Committee for PhD Matters, which consists of four PIs (Dominik Filipp, Pavel Hozák, David Staněk, Petr Svoboda) and two student representatives (Martina Huranová and Pavel Vopálenský).

Students apply to the programme through an on-line application. In 2010, nineteen candidates were selected and invited for a PhD interview. Applicants gave short English presentations of thesis research and were briefly interviewed and ranked by a three-member committee. During the interview applicants also visited several laboratories and met with group-leaders in order to find the best match. At the end, thirteen PhD students were recruited via the PhD interview procedure in 2010.

There is a number of English courses taught by scientists from the Institute: Acquisition and Processing of the Image in Microscopy (one-week practical course organized by P. Hozák), Advances in Immunology (K. Drbal, D. Filipp, R. Špíšek, P. Otáhal, T. Brdička, V. Hořejší), Advances in Molecular Biology and Genetics (two-week lecture course organized by J. Jonák and P. Svoboda), Basic Immunology (P. Otáhal, V. Hořejší), Epigenetics (P. Svoboda), Innate Immunity (D. Filipp) and RNA structure and Function (D. Staněk). PhD students also actively participate in labmeetings, journal clubs, and institutional seminars. Students can also attend English language classes, which take place directly in the IMG building to save time of PhD students.

We also aim to foster extracurricular training of our PhD students. In 2010, we started a "Welcome Weekend" for new PhD students where they are provided with basic information about the Institute and the PhD programme. In 2010, the IMG also hosted the EMBO Young Scientists Forum, which provided a number of opportunities for PhD students for career development and networking. PhD students also organize annual IMG PhD conferences and already a third conference in a row took place on 4th June 2010. With eleven student talks, a keynote lecture given by Mirka Uhlířová (Junior Group Leader, University of Cologne) and career development talk presented by Jan Peychl (Light Microscopy Facility Leader, Max Planck Institute for Molecular Cell Biology and Genetics, Dresden), the conference brought together students and other researches in an informal atmosphere and continued a nice tradition started in 2008.

Further information on PhD studies at the IMG can be found at **http://www.img.cas.cz/phd**.



Diploma Theses 2009

Ballek Ondřej Rack1 as a candidate protein involved in the regulation of translocation of Lck to lipid rafts (Supervisor: Dominik Filipp; Faculty of Science, Charles University, Prague)

Černohorská Markéta The role of JAK/STAT signalling pathway in cellular senescence (Supervisor: Zdeněk Hodný; Faculty of Science, Charles University, Prague)

Dušková Pavlína The role of DNA damage in chemically induced senescence (Supervisor: Zdeněk Hodný; Faculty of Science, Charles University, Prague)

Dzúr-Gejdošová Mária Regulation of gene expression on the model of mouse consomic strains (Supervisor: Jiří Forejt; Faculty of Science, Charles University, Prague)

Flachs Petr Construction of three congenic strains and their phenotypisation (Supervisor: Zdeněk Trachtulec; Faculty of Science, Charles University, Prague)

Hejhal Tomáš Epigenetic regulation of immunactive gene expression in tumour cells (Supervisor: Milan Reiniš; Faculty of Science, Charles University, Prague)

Kopová Ivana The role of sumoylation in premature chemically induced cellular senescence (Supervisor: Zdeněk Hodný; Faculty of Science, Charles University, Prague)

Kotáb Jan Integration preferences of avian sarcoma and leukosis viruses (Supervisor: Jiří Hejnar; Faculty of Science, Charles University, Prague)

Kovářová Denisa RNAi and reversion of Rous sarcoma virus-induced tumorigenesis (Supervisors: Jiří Hejnar, Kateřina Trejbalová; Faculty of Science, Charles University, Prague)

Machyna Martin Dual role of CD9 protein in mast cell activation (Supervisor: Petr Dráber; Faculty of Science, Charles University, Prague)

Mayer Alexandra Pathophysiological role of matrix metalloproteinase 19 in development of liver fibrosis (Supervisor: Radislav Sedláček; Faculty of Science, Charles University, Prague)

Nevařil Leonard TRAIL-induced apoptosis in populations of colon cancer cell lines under various cultivation conditions (Supervisor: Ladislav Anděra; Faculty of Science, Charles University, Prague)

Plesníková Michaela The separation, characterization and cultivation of limbal stem cells (Supervisor: Vladimír Holáň; Faculty of Science, Charles University, Prague)

Sarnová Lenka Transgenic RNAi in mouse oocytes (Supervisor: Petr Svoboda; Faculty of Science, Charles University, Prague)

Slávik Branislav Targeting of genes of interest via avian retroviral receptor Tvc (Supervisor: Radislav Sedláček; Faculty of Science, Charles University, Prague)

Svoboda Ondřej Cloning, expression and characterization of recombinant growth factors (Supervisor: Petr Bartůňek; Faculty of Science, Charles University, Prague)

Svobodová Eliška Immunomodulation properties of mesenchymal stem cells (Supervisor: Magdalena Krulová; Faculty of Science, Charles University, Prague)

Diploma Theses 2010

Auxt Miroslav — *De novo* DNA methyltransferases in the silencing of retroviral vectors [Supervisors: Jiří Hejnar, Filip Šenigl; Faculty of Science, Charles University, Prague]

Dušková Eva — Regulation of alternative splicing [Supervisor: David Staněk; Faculty of Science, Charles University, Prague]

Faltýsková Helena — Experimental models of the stem cell transfers for therapeutic purposes [Supervisor: Magdalena Krulová; Faculty of Pharmacy, Charles University, Hradec Králové]

Chudíčková Milada — Recombinant congenic strains – model for biomedical research [Supervisor: Marie Lipoldová; Faculty of Biomedical Engineering, Czech Technical Institute, Prague]

John Václav — Genes for hybrid sterility in mice: mapping of epistatic interactions [Supervisor: Jiří Forejt; Faculty of Science, Charles University, Prague]

Kašpárek Petr — Generation of a transgenic mouse model to study biological role of KLK5 in epidermis [Supervisor: Radislav Sedláček; Faculty of Science, Charles University, Prague]

Marášek Pavel — Function of paxillin complexes in cell nucleus [Supervisor: Pavel Hozák; Institute of Chemical Technology, Prague]

Pitule Pavel — Comparative genomic prediction of novel transmembrane adaptor proteins and their expression analysis [Supervisor: Karel Drbal; Faculty of Science, Charles University, Prague]

Přistoupilová Anna — Development of real-time PCR assay for detection of *Amphibiocystidium ranae* as a model approach for studying rhinosporidiosis [Supervisor: Jan Pačes; 1st Faculty of Medicine, Charles University, Prague]

Šíma Matyáš — Genetic influence on *Trypanosoma brucei brucei* infection in mice [Supervisor: Marie Lipoldová; Faculty of Science, Charles University, Prague]

Šimíček Michal — FcεRI and Kit signal to actin cytoskeleton via different pathways [Supervisor: Petr Dráber; Faculty of Science, Charles University, Prague]

Šulcová Jitka — Analysis of candidate transmembrane adaptor proteins [Supervisor: Karel Drbal; Faculty of Science, Charles University, Prague]

Těšina Petr — Structure and function of hPrp31 mutant in *Retinitis pigmentosa* [Supervisor: David Staněk; Faculty of Science, Charles University, Prague]

Vavrochová Tereza — Expression and function of molecules of innate immune system in embryonic phagocytes [Supervisor: Dominik Filipp; Faculty of Science, Charles University, Prague]

Vonková Ivana — Biochemical and functional characterization of a novel transmembrane adaptor protein Nvl [Supervisor: Tomáš Brdička; Faculty of Science, Charles University, Prague]

PhD Theses 2009

Gusareva Elena Impact of genetic and environmental factors on development of atopy and allergic diseases in Czech and Russian populations (Supervisor: Marie Lipoldová; 3rd Faculty of Medicine, Charles University, Prague)

Ivánek Robert DNA microarrays and bioinformatics in biomedical research (Supervisors: Jiří Forejt, Stanislav Kmoch; Faculty of Science, Charles University, Prague)

Krejčiříková Veronika Structural studies of mouse galectin-4 (Supervisor: Jiří Brynda; Faculty of Science, Charles University, Prague)

Růžičková Jana Eye evolution and development: an insight from jellyfish and mouse (Supervisor: Zbyněk Kozmik; Faculty of Science, Charles University, Prague)

PhD Theses 2010

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Krejčík Zdeněk Taurine-nitrogen utilized by bacteria: a diverse set of phenomena (Supervisor: Václav Pačes; Institute of Chemical Technology, Prague)

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Teaching (Semestral Courses)

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Molecular Modelling and Bioinformatics, Institute of Chemical Technology (Jiří Brynda, Pavlína Řezáčová)

Anti-tumour Immunity, Faculty of Science, Charles University (Jan Bubeník)

Immunology and Gene Therapy of Tumours, 1st Faculty of Medicine, Charles University (Jan Bubeník)

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Image Acquisition and Processing in Biomedical Microscopy, Czechoslovak Microscopy Society (Pavel Hozák)

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Model Organisms in Developmental Biology, Faculty of Science, Charles University (Zbyněk Kozmik)

Advances in Immunology of Infectious Diseases, Molecular Mechanisms of Defence against Infection, 3rd Faculty of Medicine, Charles University (Marie Lipoldová)

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Advanced Techniques in Fluorescence Microscopy, Practical Course, Institute of Molecular Genetics (David Staněk)

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Developmental Biology, Faculty of Science, Charles University (Petr Svoboda)

Advances in Molecular Biology and Genetics, annual 2-week lecture course, Institute of Molecular Genetics (Jiří Jonák, Petr Svoboda)

Innate Immunity, Faculty of Science, Charles University (Dominik Filipp)

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Seminars Biological Oxidation, 1st Faculty of Medicine, Charles University (Jiří Jonák)

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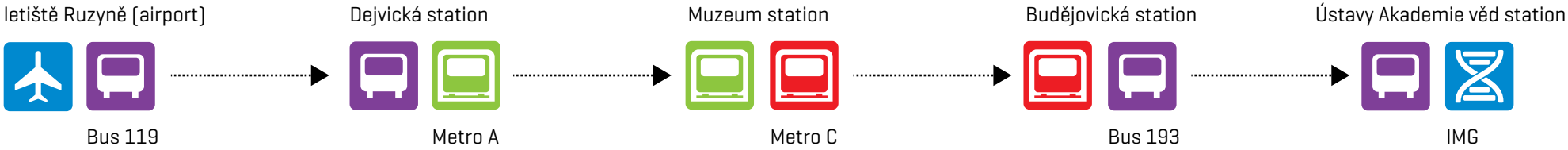

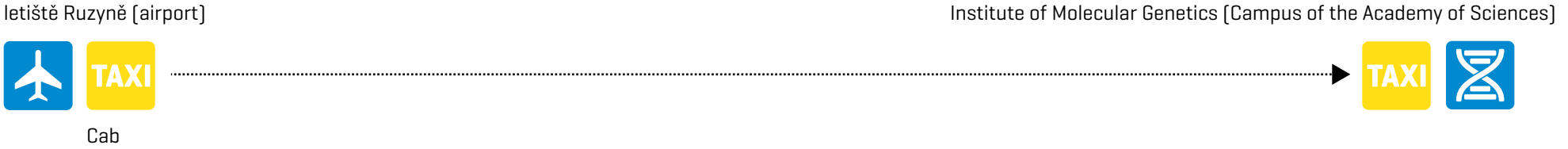


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