IMG

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

Annual Report 2011/ 2012

50th ANNIVERSARY OF THE IMG

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Introduction

In the past two years we have been successful in what is our raison d'être – good science. We have again published a number of scientific reports in peer-reviewed international scientific journals; we have 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 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 our annual Institute and PhD conferences and courses as well as other international events organized also by other on-campus institutes of the Academy.

We opened a kindergarten and gym adjacent to our main building, which are also available to our colleagues from other institutes on campus. We finished the reconstruction of our chicken breeding facility in Koleč. An extensive reconstruction of Pavilion V, which should house the new infrastructure supporting basic research in the area of chemical biology and genetics, started in 2012.

It is gratifying that our researchers repeatedly obtain prestigious local and international grants to support their experiments aiming to reveal the still abundant secrets of cells and tissues that decide on our health or disease.

At present, 22 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. Students represent a significant component of our scientific community; 80 doctoral students and 50 undergraduate students prepare their theses in our laboratories. A number of our scientists also teach at universities (e.g., eight as Professors and seven 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 collaborate with several well-prospering biotech spin-off companies that have originated at the Institute in the past years.

In 2011-12, the Institute scientists were again authors or co-authors of publications in a number of prestigious international journals (e.g. Acta Crystallographica, Blood, Current Biology, Antioxidants & Redox Signaling, Nucleic Acids Research, Cancer Research, The Journal of Immunology, Journal of Controlled Release, Carcinogenesis, Molecular and Cellular Biology, Biomolecules, International Journal of Cancer, Free Radical Biology and Medicine, Cell Cycle, Journal of Medicinal Chemistry, Journal of Biological Regulators and Homeostatic Agents, British Journal of Cancer, Molecular Biology of the Cell, Traffic, Acta Biomaterialia, The Journal of Biological Chemistry, Crystal Growth & Design, PLOS Neglected Tropical Diseases, Clinical Science, Journal of Neuropathology and Experimental Neurology, Briefings in Functional Genomics, PLOS ONE, Cellular Signalling, Methods, etc.).

The excellence of the Institute researchers is confirmed by awards and prizes. In 2011, members of the laboratory of Jiří Bartek (Zdeněk Hodný, Lenka Rossmeislová, Hana Hanzlíková, Kateřina Krejčíková, Markéta Vančurová) were awarded the Prize of the Academy of Sciences of the Czech Republic; Jarmila Hnilicová obtained the Josef Hlávka Award for the best students; Petr Heneberg received the Prize of the Czech Immunological Society for the best scientific publication by a young immunologist, and Libor Macůrek received the Otto Wichterle Award for outstanding young scientists. In 2012, Václav Pačes was honoured with the degree of Doctor Honoris Causa of the Institute of Chemical Technology, Prague.

Of crucial importance for the Institute is the BIOCEV project established within the Operational Programme Research & Development for Innovations in Vestec (www.biocev.eu). The construction of this Centre of Excellence should start in 2013 and the first of the five project programmes, Functional Genomics, was launched as the first BIOCEV research programme in August 2012.

Despite the potential funding problems, the development of our Institute continues successfully and I believe that our main goal of creating a stimulating environment for top research has already been achieved. Now we just have to make use of these good conditions.

Václav Hořejší
Director

Institute Representatives

www.img.cas.cz/about/institute-management/

Václav Hořejší
Director

Petr Dráber
Deputy Director

Jiří Špička
Deputy Director for Economy

Radislav Sedláček
Deputy Director for
BIOCEV Project Implementation

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

Vladimír Kořínek
Chairman of the Institute Council

Šárka Takáčová
Institute Secretary

Brief History

www.img.cas.cz/about/history-of-the-institute/

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 1962, 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 Ivanyi, 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.

As 1962 was the year of establishment of the IMG predecessor, IEBG, in 2012 the Institute celebrated its 50th anniversary. This anniversary was commemorated at the Annual IMG Conference and in several articles published in the Czech scientific journal Vesmír, in the Academic Bulletin and in the Czech version of the journal Scientific American.

Former Institute residence in Prague 6 - Dejvice

Former Director Jan Svoboda with the founder of the Institute and its first Director Milan Hašek

Opening of the new IMG building: Present Director Václav Hořejší with former Director Václav Pačes

New building of the Institute in Prague 4 - Krč

IMG and Its Surroundings

The Prague-Krč campus of biomedical Academy institutes

IMG is located on the campus situated in the part of Prague 4 called Krč. Five other Academy institutes share this campus – the Institute of Microbiology, Institute of Physiology, Institute of Experimental Medicine, Institute of Biotechnology and a part of the Institute of Animal Physiology and Genetics.

This arrangement allows the researchers to share common infrastructure (research core facilities, guest houses, sports areas and gym, dining halls, kindergarten). The total number of on-campus researchers and students exceeds 1200.
In close proximity to this campus there is also the Institute for Clinical and Experimental Medicine (IKEM) and Thomayer Hospital. The campus lies near a major natural park (Krč forest) and is easily accessible 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 1.2 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 four universities, the student population being more than 100.000. There are also 37 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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Research in our group is mainly focused on the analysis and characterization of signalling pathways leading to programmed death of cancer cells including cancer stem cells and uncovering mechanisms participating in the regulation of this signalling. In our major interest stands TRAIL (TNFα-Related Apoptosis Inducing Ligand), a ligand from the TNFα family capable of inducing apoptosis of transformed cells and not being harmful to the normal ones. TRAIL-induced apoptosis of sensitive cells is triggered by the interaction with its pro-death receptors TRAIL-R1/DR4 and/or TRAIL-R2/DR5. These receptors contain an α-helical protein-protein interaction domain called the death domain and together with Fas/CD95 or TNFR1 belong to the death receptor subfamily of TNFR receptors. Currently we deal with several aspects of death receptor-induced signalling such as i/ evaluation of the role of proximal signalling processes (endocytosis or endosomal acidification) in TRAIL ligand-receptor(s) Death-Inducing Signalling Complex (DISC) formation and activation of the initiator caspase-8, ii/ expression and activation of death receptors in human embryonic stem cells (hESCs) and from them derived somatic progenitors, or iii/ assessment of the effect of overexpressed/activated oncogenes such as c-myc on TRAIL- or FasL-induced apoptosis. In our collaborative projects we uncovered two novel drugs that sensitize resistant cancer cells to TRAIL-induced apoptosis. We also participate in the analysis of multiple aspects of TRAIL-induced signalling in leukaemia and colon carcinoma cells and characterization of cell death modalities in senescent normal and cancer cells. Among other death receptors we characterized posttranslational modifications and regulation of expression of the Death Receptor 6 (DR6), which is known to participate in the regulation of T- and B-cell activation and neurogenesis. We discovered that DR6 is a highly glycosylated and palmitoylated receptor, and its expression is induced upon activation of human or mouse T cells. Besides death receptors-related projects we also deal with some aspects of apoptosis- and transcription-regulating activities of chaperone adapter protein Daxx. We uncovered and characterized its functional interaction with the chromatin-remodelling ATPase Brg1, and in collaboration we investigate its possible role in the DNA damage response.

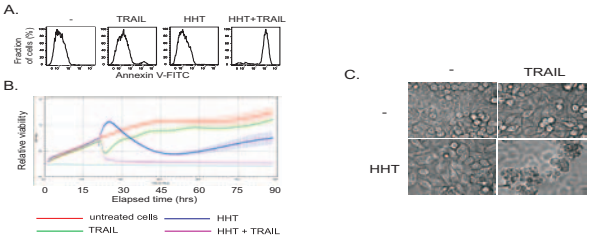

Fig. 1. Hommoharingtonine (HHT) sensitizes colorectal cancer cells to TRAIL-induced apoptosis
A. RKO cells were treated with TRAIL (20 ng/ml), 50 nM HHT and their combination for 5 hrs and apoptotic cells were quantified by Annexin-V-FITC staining and flow cytometry. B. Long-term viability of treated RKO cells analysed by xCELLigence assay. C. RKO cells treated with TRAIL, HHT and HHT+TRAIL viewed by phase-contrast microscopy.

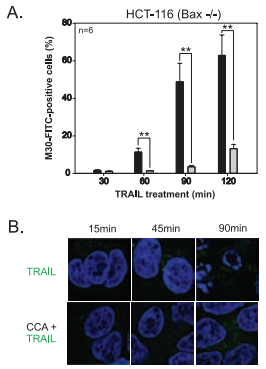

Fig. 2. Blocking endosomal acidification significantly attenuates TRAIL-induced apoptosis
A. Colorectal Bax-deficient HCT-116 cancer cells were either mock-treated (black bars) or pre-treated with 20 nM bafilomycin A1 (BafA1, grey bars), then treated with TRAIL (100 ng/ml) for indicated time periods, stained with M30-FITC (cleaved cytokeratine 18 - reflects activity of caspase-3) and analysed by flow cytometry. B. HCT-116 cells were treated with Alexa 647-labelled TRAIL (green) for indicated time periods, fixed, counter-stained with DAPI (nuclei) and visualized by confocal microscopy. BafA1 delays both aggregation of TRAIL-containing complexes in MVB and onset of apoptosis (condensed nuclei).

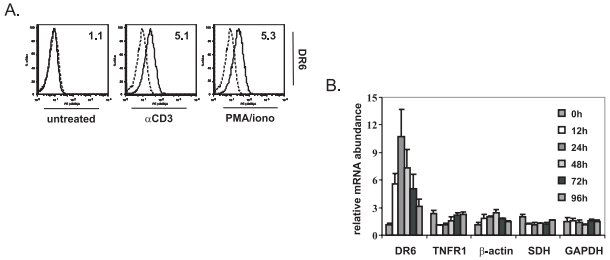

Fig. 3. Death receptor 6 (DR6) expression is induced in activated human T cells
A. Isolated human T cells were either activated by anti-CD3 crosslinking or PMA+ionomycin treatment for 48 hrs and the cell surface expression of DR6 was determined by its staining with monoclonal antibody and flow cytometry. B. Real-time qPCR analysis of the time-course of DR6 mRNA expression in PMA/ionomycin-treated human T cells and comparison with TNFR1 expression; b-actin, SDH and GAPDH serve as **housekeeping genes.**

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From the left:
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Laboratory of Genome Integrity

DNA damage response, cytokines, cellular senescence, cancer, ageing

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Realization of complex tasks of living organisms depends on the information stored in DNA of their genomes. The loss of this information due to endogenous and exogenous physicochemical damage to DNA results in disintegration of homeostasis at the cellular and organism levels manifested as diseases, including cancer and ageing. Several tightly orchestrated mechanisms take care of preserving the intactness of genetic information by preventing and repairing DNA damage. Our research is centred on cellular responses (collectively termed 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 radioresistance and chemoresistance of cancer cells; 3) mechanisms of cellular response to persistent irreparable DNA damage lesions manifested as irreversible cell cycle arrest (cellular senescence); 4) role of DNA damage-induced expression of secreted factors (cytokines) in autocrine/paracrine signalling and intercellular communication; and 5) impact of the above mechanisms on cancer and ageing with the aim to find new therapeutic approaches, such as thermotherapy using targeted gold nanoparticles. To summarize our main recent findings, we have functionally characterized two proteins (UBA1 and Nup153) identified previously during high-throughput siRNA-based phenotypic screening of factors involved in posttranslational modifications of DDR components. We have found that the IL6-STAT3 signalling pathway is involved in transcription regulation of PML tumour suppressor via autocrine/paracrine mechanisms. We have found that persistent DNA damage and resulting cellular senescence is transmittable to surrounding cells via factors secreted from damaged cells (so-called bystander senescence). Currently, we are characterizing mechanisms of radioresistance and chemoresistance of prostate metastatic cancer cells and we are screening DNA and RNA aptamers utilisable for targeting of gold nanorods to cancer cells by the use of a selection procedure based on Cell-Selex.

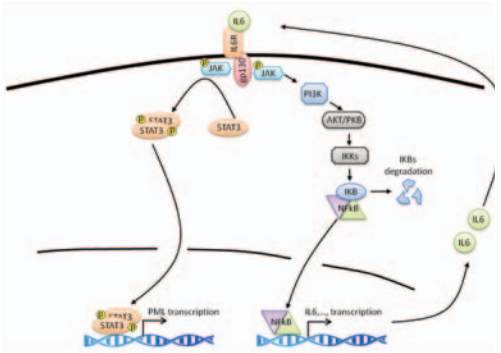

Fig. 1. Role of autocrine/paracrine IL6/STAT3 and NFκB signalling pathways in transcription regulation of tumour suppressor PML.

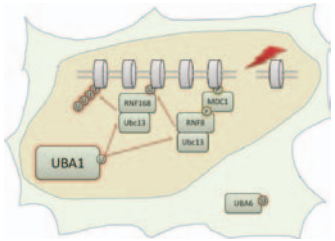

Fig. 2. Involvement of UBA1 in ubiquitination of factors recruited to the site of DNA double-strand breaks.

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Laboratory of Cell Differentiation

Haematopoietic and neural cell differentiation, zebrafish development, nuclear receptors, chemical biology

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. 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 and lipid distribution. We proposed that DISP3 represents a new molecular link between thyroid hormone and cholesterol metabolism in the brain (Zikova et al. 2009). In addition, we have identified two neural stem cell lines that highly express Disp3. 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. To better understand the role of Disp3 in vivo we have established mouse transgenic lines overexpressing Disp3 in astrocytes (GFAP promoter) and oligodendrocytes (PLP promoter). Analysis of these mice demonstrates the important role of Disp3 in lipid homeostasis in neural cells in vivo.

We have extended our studies on vertebrate haematopoietic development by introducing a new model organism in our laboratory – the zebrafish – and we have established ex vivo cultures of haematopoitic cells (Stachura et al. 2009). Recently, we have produced several recombinant zebrafish growth factors (Epo, Gcsf, Tpo) that allow us to establish, for the first time, zebrafish haematopoietic clonal assays in semisolid media (Stachura et al. 2011, Svoboda et al., submitted). Moreover, these tools enabled us to reveal the clonogenic and proliferation capacity of bi-potent thrombo/erythropoietic progenitors with respect to their mammalian hematopoietic counterparts. Despite obvious phenotypic differences between fish and mammalian thrombocytes and erythrocytes, our results strongly demonstrate the evolutionary conservation of basic processes and molecular mechanisms of erythro/thrombopoiesis in the vertebrates.

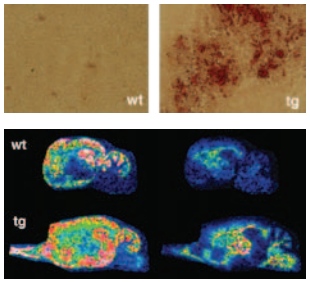

Fig. 1. Disp3 regulates lipid homeostasis in vivo
(A) Lipid accumulation in lipid droplets in PLP-Disp3 transgenic animals. Oil red staining of sagittal section of mouse cortex. (B) Distribution of various sfingolipids in wt and PLP-Disp3 transgenics as revealed by MALDI imaging of sagittal sections of mouse brain.

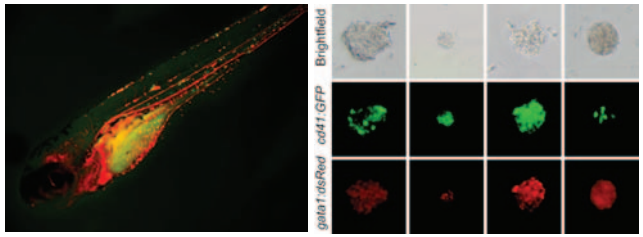

Fig. 2. Zebrafish as a model to study vertebrate hematopoiesis
(A) Double hemizygous transgenic zebrafish Tg(gata1::DsRed); Tg(cd41::EGFP) at 4 days post fertilization with single hematopoietic cells fluorescently labelled (red, erythroid cells, green, thrombocytes). (B) Hematopoietic cells derived from zebrafish whole kidney marrow (WKM) were cultivated ex vivo in semisolid media (methocel) in the presence of recombinant zebrafish thrombopoietin (TPO) and erythropoietin (Epo), giving rise to bi-potent thrombo/erythropoietic progenitors.

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- Ministry of Industry and Trade of the Czech Republic, FR-TI4/802 - Development of new chemical compounds with anti-tumor activities or use in regenerative medicine, 2012-2015, P. Bartůněk
- TA CR, TA02010212 - ReceptorX: Integrated platform for drug discovery and development, 2012-2015, P. Bartůněk
- Operational Programme Prague – Competitiveness, CZ.2.16/3.1.00/28026 - Label-free Technology Platform, 2012-2013, P. Bartůněk
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Laboratory of Molecular Pharmacology

G-protein-coupled receptors, neurotransmitters, metabotropic glutamate receptors

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Glutamate is a major excitatory neurotransmitter that activates two types of receptors in mammalian brains: ligand-operated ion channels (NMDA, AMPA and kainate receptors) and metabotropic G-protein-coupled receptors (GPCRs). There are eight genes that code for the metabotropic glutamate (mGlu) receptors in mammals. These diverge in the location within brain regions and cellular compartments and have distinct functional properties. As such, they constitute a promising target for treatment of certain neurological diseases.

Our research is focused on the structure-function relationship of these receptors. The mGlu receptors belong to family C GPCRs and are traditionally viewed as homodimers composed of two identical subunits. Using the mutagenesis approach combined with a functional expression system we showed that within their homodimeric complexes only one subunit reaches the active state. Our recent data using the dynamic FRET approach are in accord with this notion. The activation process of these family C GPCRs is initiated by agonist binding that causes conformational changes of the extracellular ligand-binding domain. This is followed by relative movement of the transmembrane regions of the two subunits, and finally a conformational change within one of the heptahelical transmembrane domain can be transmitted to the intracellular signalling machinery. The active state of these receptors is thus asymmetrical.

Recent data from our laboratory suggest that splice variants of the mGluRs can also form heterodimers. We have obtained data suggesting that heterodimerization between distinct splice variants of the mGlu1 receptor, mGluR1a and mGluR1b, results in novel receptor complexes with modified trafficking properties in transfected heterologous cells and primary neurons. This observation about the combination of distinct splice variants within the dimeric receptor complexes are being investigated from the point of functional relevance in vivo.

Fig. 1. Activation of Class C GPCR schematically
Together with our collaborators we brought evidence [**EMBO J 2005** 24(3): 499-509 and **Science Signal 2012** 5(237): ra59] that activation of metabotropic glutamate receptors after agonist binding (red rectangle) within extracellular binding sites (also known as Venus fly-trap like domains) results in the change in relative position of the two subunits of transmembrane (heptahelical) domains followed by a conformational change within one of the two heptahelical regions. This active state (yellow star) of a single heptahelical domain then may activate intracellular G-protein signalling and possibly other pathways.

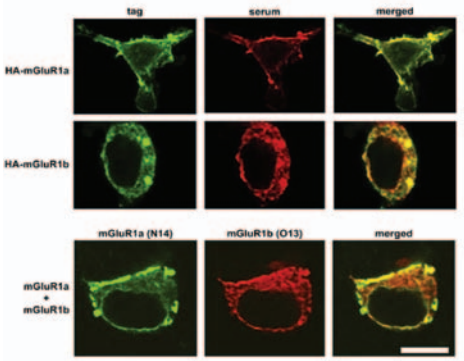

Fig. 2. For immunocytochemistry cells were transfected with HA-mGluR1a and stained with monoclonal anti-HA antibodies (secondary antibodies labelled with FITC) and our N14 antibodies (secondary anti-rabbit antibodies labelled with Cy3). c-Myc mGluR1b-expressing cells were labelled with anti-c-Myc antibodies and guinea pig anti-mGluR1b antibodies (013) 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 10 mm *in vivo*.

- GA CR, GA303/08/1591 - Study of glutamate receptors conformational changes using novel fluorescent techniques, 2008-2012, J. Blahoš
- GA CR, GAP303/12/2408 - Functional consequences of metabotropic glutamate receptor 1a and 1b splice variants assembly in heterodimeric complexes in vivo, 2012-2016, J. Blahoš

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Laboratory of Biology of Cytoskeleton

Modulation of microtubule organization, microtubule proteins, γ-tubulin, 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 TIRF microscopy, live cell imaging and quantification of MT plus end dynamics. Our results demonstrate that Ca2+ plays an important role in the regulation of MT organization in activated mast cells. We have also shown that γ-tubulin, which is assumed to be a typical cytosolic protein, is also present in nucleoli of mammalian interphase cells of diverse cellular origin. Nuclear γ-tubulin associates with tumour suppressor protein C53 and modulates DNA damage G2/M checkpoint activation. We have demonstrated that even though γ-tubulins are differentially expressed during mouse embryogenesis and in adult tissues, they are functionally redundant with respect to their nucleation activity. Finally, we have shown that MT-severing ATPase spastin is overexpresed in glioblastoma multiforme, the most common and deadliest form of primary brain cancers, and that spastin expression might be linked to tumour cell motility, proliferation and invasion.

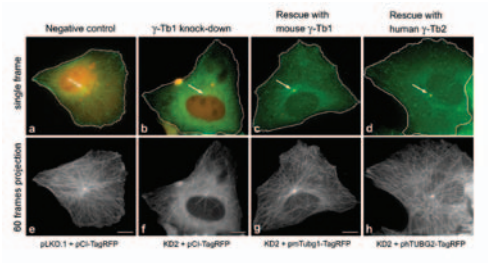

Fig. 1. γ-Tubulin 2 rescues microtubule formation in γ-tubulin 1-depleted cells. Time-lapse imaging of U2OS-EB1 cells for quantitative evaluation of microtubule (+) end dynamics. Cells with depleted γ-tubulin 1 (KD2) expressing either TagRFP (pCI-TagRFP), mouse γ-tubulin 1 (pmTubg1-TagRFP) or human γ-tubulin 2 (phTUBG2-TagRFP). (a-d) Still images of typical cells selected for evaluation. (e-f) Maximum intensity projections of 60 consecutive time-frames from acquired time-lapse sequences. Microtubule track density is rescued in cells expressing exogenous mouse γ-tubulin 1 (g) or exogenous human γ-tubulin 2 (h).

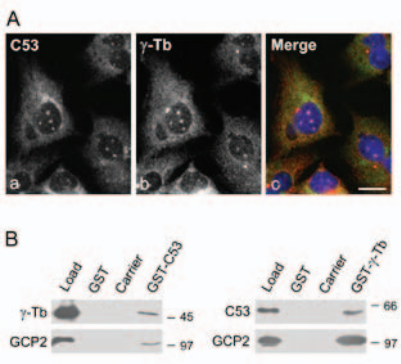

Fig. 2. Association of nucleolar γ-tubulin with tumour suppressor protein C53. (A) Nucleolar localization of C53 and γ-tubulin. Cells were double-label stained with antibodies to C53 (a; green) and γ-tubulin (b; red). DAPI (c; blue). (B) Interaction of γ-tubulin with C53 in GST pull-down assay. Lysates (Load) were incubated with immobilized GST alone (GST), beads used for immobilization (Carrier) or immobilized GST-fusion proteins (GST-C53; GST- γ-tubulin). Blots of bound proteins were probed with antibodies to γ-tubulin (γ-Tb), C53 and γ-tubulin-associated protein GCP2.

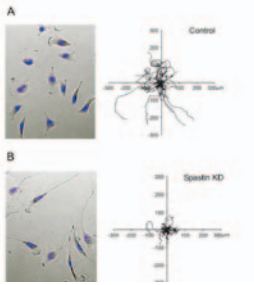

Fig. 3. Effect of spastin depletion on morphology and migration of glioblastoma T98G cells. (A) Control cells. (B) Spastin-depleted cells (spastin KD). Still images from time-lapse imaging; combination of bright field and fluorescence of nuclei. Randomly chosen time-lapse sequences were analysed and all tracks were aligned, with their starting points at coordinate position [0,0].

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- GA CR, GAP302/10/1701 - Analysis of functional differences between γ-tubulins, 2010–2012, E. Dráberová
- GA CR, GPP302/11/P709 - Analysis of microtubular changes during activation of mast cells, 2011–2013, V. Sulimenko
- TA CR, TA01010436 - New generations of DNA aptamers, 2011–2013, Pe. Dráber, Pa. Dráber
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- AS CR, M200521203 – Regulatory proteins of microtubules: new target molecules for therapy of brain tumours, 2012–2015, P. Dráber
- GA CR, GAP302/12/1673 – Role of γ-tubulin complexes in regulation of cell cycle, 2012–2015, P. Dráber
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Laboratory of Signal Transduction

Plasma membrane signalosomes, immunoreceptor signalling, mast cell activation, chemotaxis

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The main research interest of our laboratory has been traditionally focused on understanding molecular mechanisms governing signal transduction from the high affinity IgE receptor (FcεRI) and other plasma membrane receptors to the cytoplasm. We continued in functional studies on the role of transmembrane adaptor proteins in regulation of such important antigen-mediated events as calcium response, degranulation and chemotaxis. Especially, we attempted to solve the problem we noticed in our previous studies that NTAL has a negative regulatory role on FcεRI activation in mast cells isolated from NTAL knockout mice, but positive regulatory role in human mast cells with NTAL knockdown (through RNA interference). We found that the observed discrepancies did not reflect compensatory developmental alterations in mouse cells, as expected, but rather different roles of NTAL in FcεRI-induced signalling pathways in human and mouse cells. To gain more insight into NTAL function, we also examined gene expression profiles in resting and antigen-activated mast cells with NTAL knockout and knockdown and corresponding controls and we identified several genes that were differentially expressed in NTAL deficient and wild-type cells. We also continued in studies on stromal interacting molecule 1 (STIM1) in mast cell signalling and found that STIM1 is required for formation of microtubule protrusions in antigen-activated cells and for chemotaxis towards antigen.

Several other molecules were analysed (Csk, PAG, STAT5, CD9, ORMDL3, FcεRI) to get better insight into the signalling pathways involved in mast cell signalling. Significant effort was devoted to identifying new signalling molecules by high-throughput screening using RNA interference libraries.

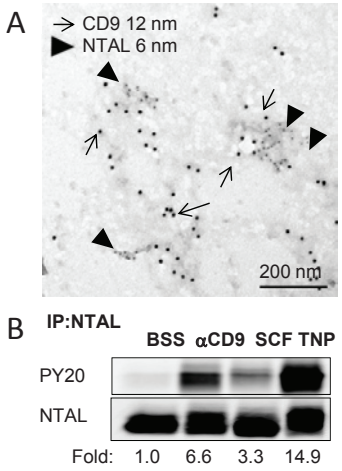

Fig. 1. CD9-NTAL crosstalk. A. Membrane topography of tetraspanin CD9 and transmembrane adaptor protein NTAL on isolated plasma membrane sheets as detected by electron microscopy. B. phosphorylation of NTAL after activation of mast cells with CD9-specific antibody, stem cell factor (SCF) and antigen (TNP) as detected by immunoblotting of immunoprecipitated NTAL.

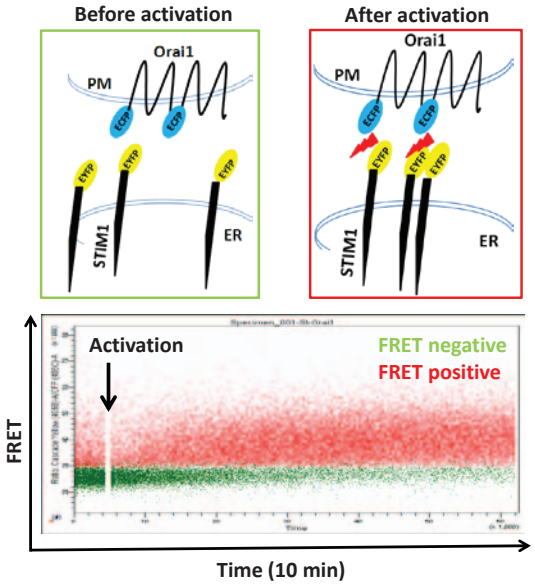

Fig. 2. Dynamics of STIM1-Ora1 crosstalk. Interactions between STIM1 and Orai1 were determined by fluorescence resonance energy transfer (FRET). FRET (in red) occurs between STIM1-EYFP and Orai1-ECFP in cells after activation with thapsigargin that releases calcium from endoplasmic reticulum. FRET is determined by flow cytometry.

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- GA CR, GAP302/10/1759 - Function-structure relationships between transmembrane adaptor-based signalosomes in mast cells, 2010-2013, L. Dráberová
- Ministry of Industry and Trade of the Czech Republic, FR-TI3/067 - Genetically modified polymerases and their use for amplification of unpurified DNA, 2011-2014, Pe. Dráber
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Laboratory of Molecular Virology

Carcinogenesis, tumour microenvironment, metastasis, myofibroblast, photodynamic therapy

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The research efforts of the group focus on genes and molecular mechanisms involved in 1) initiation, promotion, and progression of experimental malignancies; 2) metastasis and interactions between a tumour and its microenvironment; 3) targeted intervention into pathological cell communication and induction of cell death in tumour cells with synthetic ligands; 4) fibrosis-related biology of myofibroblasts; 5) fate determination in multipotent neural cells and differentiation of myogenic precursors. In studies of cell fate determination, differentiation and malignant transformation of haematopoietic and neural cells and myogenic precursors, myb genes are used as molecular tools. In studies of genes involved in the formation of kidney, liver and lung tumours in chicks, insertional mutagenesis by MAV retroviruses is exploited. Driver mutations induced by proviral insertions are identified and analysed. Genes of the egr family serve as tools to study metastatic potential of experimental tumours. These genes are also used to study the TGF-β signalling network in myofibroblasts. Attempts are made to influence profibrotic properties of myofibroblasts. Various chemical compounds designed and synthesized by the cooperating group (Institute of Chemical Technology, Prague) are used as ligands targeting cell receptors and interfering with cell signalling. Porphyrin derivatives accumulating preferentially in tumours are applied as photosensitive compounds and drug delivery vehicles in multimodal therapy combining chemotherapy, photodynamic therapy and immunotherapy to potentiate cell death in cancer cells and tissues. Attempts are made to define subcellular targets and mechanisms of action of promising photosensitive compounds.

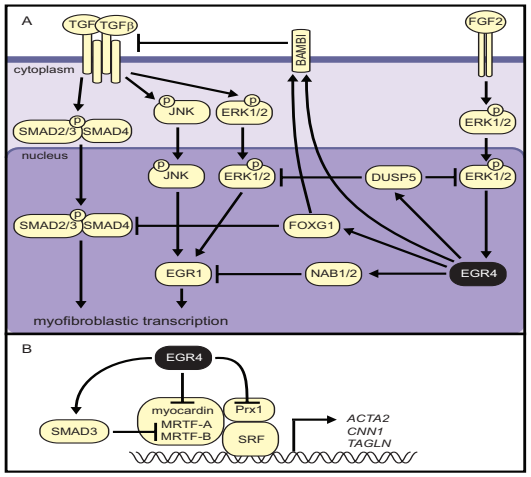

Fig. 1. Proposed mechanism of action of constitutively expressed EGR4. (A) Arrangement of the signalling pathways involved in myofibroblastic differentiation and how genes upregulated by EGR4 can interfere with these pathways. FOXG1 interferes with the activity of SMAD proteins, BAMBI negatively regulates signalling through TGF-β receptors, DUSP5 inactivates ERK kinases, and NAB1 and NAB2 proteins repress EGR1-dependent transactivation of myofibroblastic phenotype-related genes. For the sake of simplicity, other signalling pathways activated by TGF-β and FGF2 signalling are not shown, nor are effectors of pathways other than EGR1 and EGR4. (B) Several effects can underlie the ability of EGR4 to downregulate expression of the alpha smooth muscle actin-containing contractile apparatus in myofibroblasts. Indirect stabilization of the SMAD3 protein can elicit repression of the activity of MRTF proteins. Downregulation of the expression of myocardin and Prx1 would decrease transactivation of SRF-regulated genes.

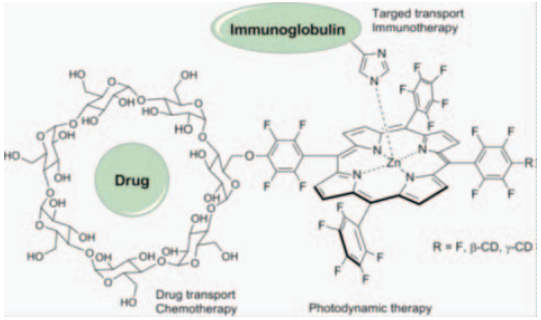

Fig. 2. Multimodal therapeutic system combining chemotherapy, photodynamic therapy and immunotherapy. The targeted drug delivery for cytostatics is ensured by the drug carrier Zn-porphyrin-cyclodextrin conjugates and their supramolecular coordination complexes with immunoglobulins or therapeutic proteins.

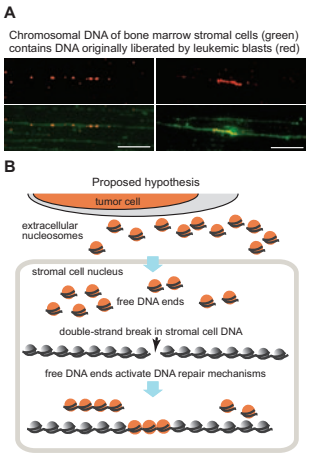

Fig. 3. Nucleosomes liberated by tumour cells enter nuclei of neighbouring cells and thus can damage cells of the tumour microenvironment by mutagenesis or cell death induction. (A) DNA combing technique reveals the integration of the BrdU-labelled nucleosomal DNA released from blasts of experimental acute monoblastic leukaemia (red staining in upper panels) within chromosomal DNA of bone marrow stromal cells (green). Lower panels show overlays of red and green staining. (B) A hypothesis: Free DNA ends of nucleosomes produced by tumour cells induce a DNA damage response (DDR) and DNA repair in stromal cells. This results in higher mutation rate in tumour stroma. High intranuclear concentrations of tumour cell DNA fragments induce apoptosis in recipient stromal cells.

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Immune tolerance, TLRs in embryonic haematopoiesis, TCR signalling

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The main theme of our research is the understanding of the mechanisms guiding the process of central and peripheral tolerance. Specifically, our research is focused on the contribution of cellular and molecular factors to the onset and maintenance of diabetes [1,2]. In collaboration with clinical laboratories we were able to demonstrate increased levels of myeloid α-defensin expression in the capillary blood of recently diagnosed patients with diabetes [3]. We have extended these analyses to the question related to the physiological role of enteric α-defensin production in the thymus and the maintenance of central tolerance in the small intestine. We showed that these molecules expressed by Paneth cells in the crypts of the small intestine are also expressed by a sizable fraction of medullary thymic epithelial cells (mTECs) [Fig. 1], where their expression is dependent on the AIRE transcription regulator. The immunological consequences of defective enteric α-defensin expression in the thymus were confirmed by the presence of anti-HD5 autoantibodies in the sera of APECED patients deficient in AIRE function. This seropositivity highly correlated with the clinical manifestation of idiopathic diarrhea. In addition, we demonstrated a strong correlation between the severity of intestinal damage and the multiplicity of intestinal immunoreactivities in the serum of APECED patients [Fig. 2]. As GI symptoms and their underlying pathogenesis in APECED patients are still poorly understood, these findings provide new insight into the process of maintenance of central tolerance in the small intestine [manuscript submitted]. In addition, we have started to characterize functionally distinct stromal cell populations present in lymph nodes [Fig.3].

We are also very interested in the expression pattern of Toll-like receptors and other TIR domain-containing immune-related proteins during the early mammalian embryogenesis. Data accumulated so far point to the spatially and temporarily strictly regulated expression of these receptors. Surprisingly, Toll-like receptor 2 seems to be a suitable surface marker which allows tracking the earliest haematopoietic progenitors in a precirculation embryo [manuscript submitted].

We also continue in our effort to understand the very early biochemical events leading to the activation of T cells. 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 lipid rafts. Interestingly, we showed that a small pool of kinase active Lck in primary naive CD4+ T cell is maintained in a special type of lipid microdomains, called 'heavy lipid rafts' [4,5][Fig. 4].

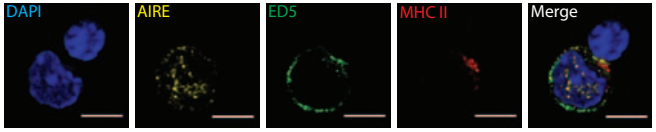

Fig. 1. Confocal immunofluorescence images of enteric defensin-positive thymic stromal cell [ED5, green] interacting with a thymocyte. The cells were co-stained with AIRE [yellow] and MHCII [red]. Nuclei are stained by DAPI [blue]. White bar represents 5-μm scale.

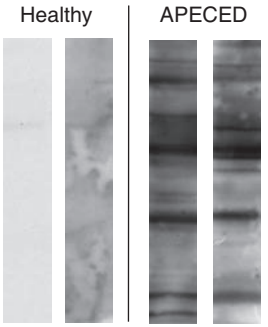

Fig. 2. Western blot analysis of sera derived from APECED patients and controls showed increased numbers of autoantibodies against intestinal antigens in the former. Representative images from two patients from each group are shown.

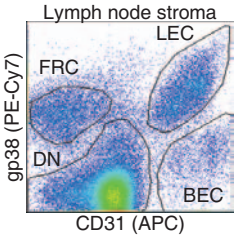

Fig. 3. Distinct immunophenotypes of stromal cell populations present in lymph nodes revealed by staining with gp38 and CD31 surface antigens.

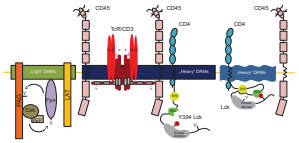

Fig. 4. A new model of TCR triggering incorporates 'heavy' DRMs as a structurally and functionally important component of cellular membranes. For detail see [4,5].

- GA 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 [co-applicant]
- Ministry of Defence of the Czech Republic, 0801 8 8110 R - Study of novel prophylactic agents against Francisella tularensis infection, 2008-2011, D. Filipp [co-applicant]
- GA CR, GA310/09/2084 - Characterization of the molecular machinery regulating the recruitment of signaling molecules to lipid rafts, 2009-2013, D. Filipp
- GA CR, P302/12/6101 - Molecular mechanisms of signaling through leukocyte receptors – their role in health and disease, 2012-2017, D. Filipp [co-applicant]

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Matuš Vobořil, BSc / Diploma Student (since 2012) · Martina Benešová, MSc / Research Assistant · Ondřej Ballek, MSc / PhD Student

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Laboratory of Mouse Molecular Genetics

Mouse genomics, hybrid sterility, *Prdm9*, meiotic silencing, chromosome substitution strains

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We identified the first vertebrate hybrid sterility gene *Prdm9* (Meisetz), encoding a meiotic histone H3 lysine-4 tri-methyltransferase. Positional cloning was confirmed by a rescue experiment using the intact *Prdm9* transgene in bacterial artificial chromosomes with the "fertility" *Hst1*^{f} allele. Identification of the *Prdm9* hybrid sterility gene reveals a role for epigenetics in speciation, and opens a window to a systems approach to the hybrid sterility gene network. To characterize the incompatibilities underlying hybrid sterility, we phenotyped reproductive and meiotic markers in males with altered copy numbers of *Prdm9*. A partial rescue of fertility was observed upon removal of the B6 allele of *Prdm9* from the azoospermic (PWD x B6)F1 hybrids, whereas removing one of the two *Prdm9* copies in PWD or B6 background had no effect on male reproduction.

Chromosome substitution, or consomic strains C57BL/6J-Chr # PWD/Ph/ForeJ, recently constructed in our laboratory, are used for dissecting the genomic architecture of sterility of *Mus m. musculus* x *Mus m. domesticus* hybrids. They are also employed in phenome analysis in collaboration with The Jackson Laboratory, Bar Harbor, Maine, USA (Dr. K.L. Svenson). 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.

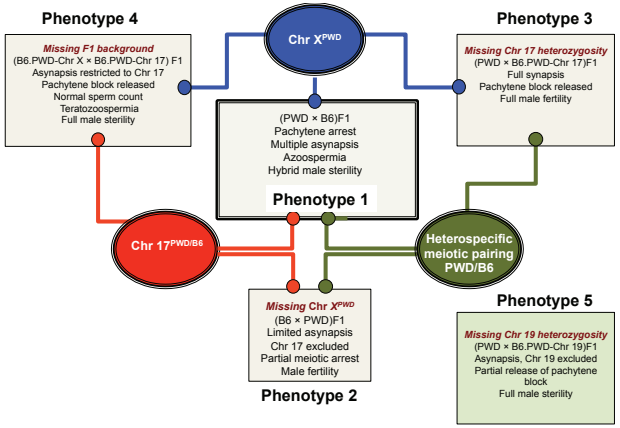

Fig. 1. Genetic architecture of hybrid sterility of (PWD x B6)F1 hybrids. Five basic hybrid sterility phenotypes are controlled by three major genomic determinants. Interaction of all five components is required to attain full pachytene block.

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- GA CR, GAP305/10/1931 - Identification of interactors of the meiotic histone methyltransferase Hybrid sterility 1 (PR-domain 9), 2010-2013, Z. Trachtulec
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Hana Vágnerová / Secretary · Václav Gergelits / Diploma Student (since 2012) · Ondřej Mihola, PhD / Postdoctoral Fellow · Mária Dzúr-Gejdošová, MSc / PhD Student · Petr Jansa, PhD / Research Fellow

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Laboratory of Viral and Cellular Genetics

Receptors for retroviruses, retroviral vectors, endogenous retroviruses, silencing of retroviruses, epigenetics

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Retroviruses multiply through a complex replication cycle in their host cells. They enter cells via specific receptors displayed at the cell surface, integrate into the host chromosomes, and use the cell transcription and proteosynthesis machineries to express retroviral structural or enzymatically active proteins. At multiple levels, cellular restriction factors regulate retroviral replication. Specific binding of retroviral envelope proteins to host cell receptors is the prerequisite for cell permissiveness to the infection. Retroviruses can broaden their host range by mutations of the env gene, and vice versa, host cells develop resistance to retroviruses by mutations of genes encoding the specific receptors. We have described such an interesting semi-resistant phenotype in chicken line P and in red jungle fowl and explained it by intronic mutations of the receptor Tva. Both mutations decrease the splicing efficiency of tva transcripts and diminish the display of receptor molecules. 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 might be an obstacle in the case of retroviral vectors used for the gene transfer in gene therapy trials. We have demonstrated that vectors derived from avian sarcoma and leukosis viruses are efficiently silenced through DNA methylation and de novo DNA methyltransferase Dnmt3b plays a crucial role in this process. The epigenomics of retroviral integration sites revealed that only proviruses localized close to the transcription starts of targeted genes keep long-term transcriptional activity without provirus silencing and promoter methylation. The protective region around the transcription start site is marked by enrichment in H3K4 trimethylation. In gene bodies, out of H3K4me3 islands, the proviruses are silenced progressively with the distance from transcription start and in intergenic regions, the proviruses are efficiently silenced. Another example of epigenetic regulation is represented by endogenous retroviruses in the human genome. Fusogenic envelope glycoproteins encoded by two copies of HERVs, ERVWE1 and ERVFRDE1, are strictly placenta-specific, and their expression in other tissues must be prevented by DNA methylation and histone methylation. Another level of syncytin control, at least in syncytin-1, is splicing of retroviral RNA, which has been observed in trophoblastic cells and aberrantly in male germ line tumours. The possible role of hydroxymethyl cytosine in transcriptional regulation of both infectious and endogenous retroviruses is under study.

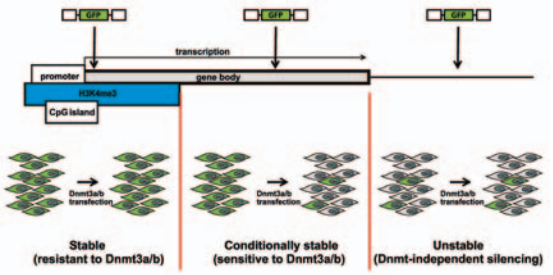

Fig. 1. Expression of proviruses integrated in different genomic localizations, an integrative model. Proviruses integrated close to the TSS within the H3K4 trimethylation region are stably expressed and insensitive to overexpression of de novo Dnmt (left). Proviruses integrated within the gene bodies outside of the H3K4me3 regions are silenced but their stable expression remains in the absence of Dnmt3a/Dnmt3b (conditionally stable expression; middle). Intergenic insertions result in rapid silencing of proviral expression, which is independent of de novo Dnmts (right). Provirus expression is indicated by a picture with green and gray cells.

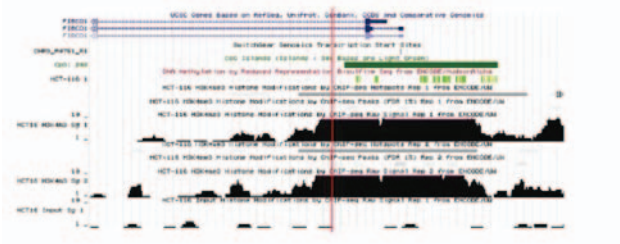

Fig. 2. Example of an integration site with stable provirus expression. The targeted gene (blue), integration site (vertical red line), CpG island (dark green), distribution of CpG dinucleotides (light green), and profile of H3K4 trimethylation (black) are shown.

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- Ministry of Agriculture of the Czech Republic, QJ1210041 – New type of vaccine against chicken viral diseases, 2012-2016, J. Hejnar
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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 particularities of immunity in the eye. Since successful treatment of a severely damaged ocular surface 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.

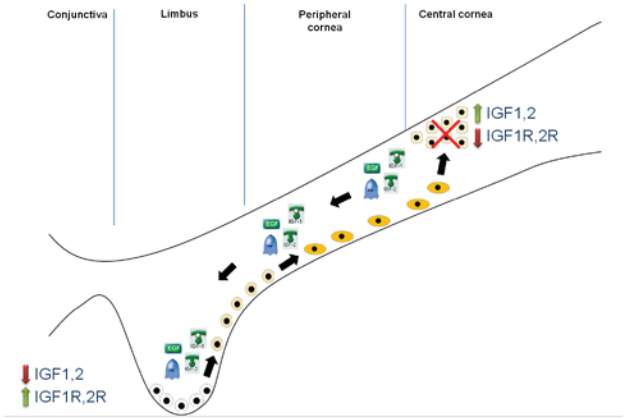

Fig. 1. Scheme of the insulin-like growth factor-I (IGF-I) signalling after the damage of the cornea.

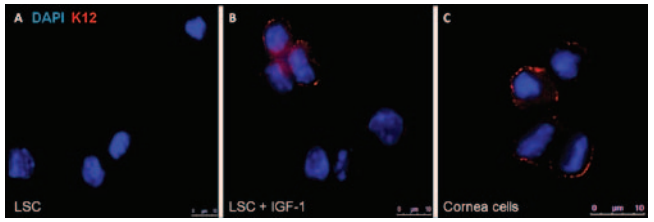

Fig. 2. Immunostaining for cytokeratin K12 protein in limbal cells treated with IGF-I. A single cell suspension prepared from the limbal tissue of control mice was incubated for 72 h with or without IGF-I, and the cells were stained with a goat antibody against mouse K12. The nuclei were stained with DAPI (blue). The limbal cells were cultured without IGF-I (A) or with IGF-I (B). As a positive control were used cultured corneal epithelial cells (C).

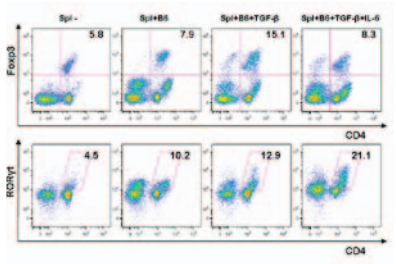

Fig. 3. The regulatory effects of TGF-β and IL-6 on Foxp3 and RORγt expression in spleen cells stimulated with alloantigens. Spleen cells from BALB/c mice were cultured unstimulated, stimulated with irradiated B6 spleen cells, or were stimulated with B6 cells in the presence of 2 ng/ml of TGF-β or TGF-β together with 10 ng/ml of IL-6. The percentage of CD4+Foxp3+ or CD4+RORγt+ cells was determined by flow cytometry.

- AS CR, KAN200520804 - Biocompatible nanofiber scaffolds forming novel drug matrices for the application of biologically and pharmacologically active substances, 2008-2012, V. Holáň
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- GA CR, GA206/08/0640 - Host-parasite relationship, hybrid zone, immune system, house mouse, Mus musculus, 2008-2012, V. Holáň
- GA CR, GAP301/11/1568 - Cellular aspects of transplantation tolerance, 2011-2014, V. Holáň
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Transmembrane adaptor proteins, membrane microdomains, Csk, immunoreceptor signalling

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In recent years the major topics of our laboratory have been (1) leukocyte membrane microdomains and (2) leukocyte membrane associated signalling molecules, namely several novel transmembrane adaptor proteins and their involvement in immunoreceptor signalling. In 2011-2012 we continued our studies on several novel raft-associated transmembrane adaptors (LST1A, PRR7, SCIMP), functional effects of targeting protein tyrosine kinase Csk into various membrane compartments, and on receptor phosphatase CD148. Furthermore, we produced a number of novel monoclonal antibodies as valuable research and potentially diagnostic tools, e.g. those to Drebrin, OPAL1, TFG, PCLO, ARH GEF, DDIT, AGPS.

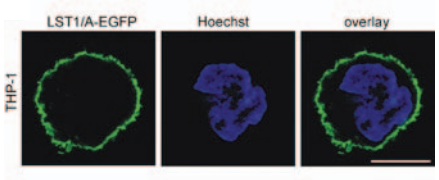

Fig. 1. Plasma membrane localization of transmembrane adaptor protein LST1/A. THP-1 cells were stably transfected with LST1-EGFP, fixed, permeabilized and nuclei were stained with Hoechst 33258. *(Draber et al. J Biol Chem 2012; 287: 22812)*

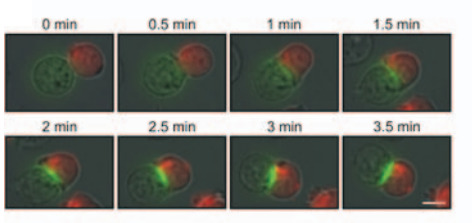

Fig. 2. Translocation of transmembrane adaptor protein SCIMP into immunological synapse. Ramos cells transfected with SCIMP-GFP (green) were loaded with superantigen and subsequently Jurkat T cells (labeled by DDAO in red) were added. Immunological synapse formation was observed by live cell imaging. . *(Draber et al. Mol Cell Biol 2011; 31: 4550)*

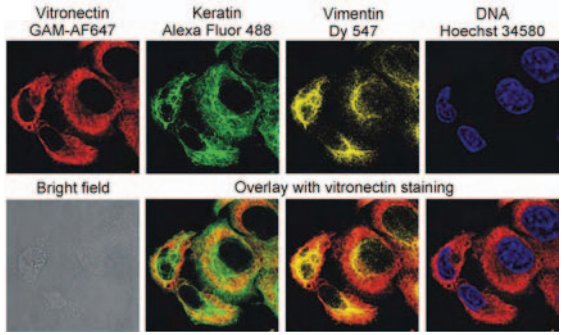

Fig. 3. Vitronectin-binding cytoplasmic components are not intermediate filaments. HeLa cells cultivated on microscope cover slips were fixed and permeabilized. The cells were incubated in 50% human serum, stained with anti-vitronectin and GAM-Alexa Fluor 647 antibodies, followed by anti-pan cytokeratin-Alexa Fluor 488 and anti-vimentin-Dy 547 antibodies in 20% mouse serum and Hoechst 34580 staining. *(Stepanek et al. PloS One 2011; 6: e19243)*

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- 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 - Signaling and adaptor proteins of leukocyte membrane microdomains, 2009-2012, V. Hořejší
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- GA CR, GAP302/12/1712 – Function of Csk binding proteins SCIMP and PSTPIP2 in leukocyte signalling and inflammatory responses, 2012-2015, T. Brdička
- GA CR,GAP302/12/G101 – Molecular mechanisms of leukocyte receptor signalling – their roles in health and disease, 2012-2018, V. Hořejší
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From the left:
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Not in the picture:
Karel Drbal, PhD / Research Fellow · Pavel Otáhal, MD, PhD / Research Fellow · Markéta Kucová / Technician (maternity leave) · Tereza Skopcová / Technician (maternity leave) · Ladislava Sobotková / Technician (until 2011) · Peter Dráber, MSc / PhD Student (until 2012) · Matouš Hrdinka, MSc / PhD Student (until 2011) · Tereza Ormsby, MSc / PhD Student (until 2011) · Ondřej Štěpánek, MSc / PhD Student (until 2012) · Šimon Borna / Diploma Student (since 2012) · Klára Kotlabová / Diploma Student

Laboratory of Biology of the Cell Nucleus

Cell nucleus, gene expression, nucleoskeleton, nuclear actin, myosins and lipids, microscopy, ultrastructural methods

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In diploid mammalian cells, some 6 x 10^9 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 contributes to the nuclear compartmentalization, (3) functions of nuclear myosins and actin in transcription and gene expression, (4) functions of nuclear lipids, (5) development of new microscopy methods for ultrastructural studies.

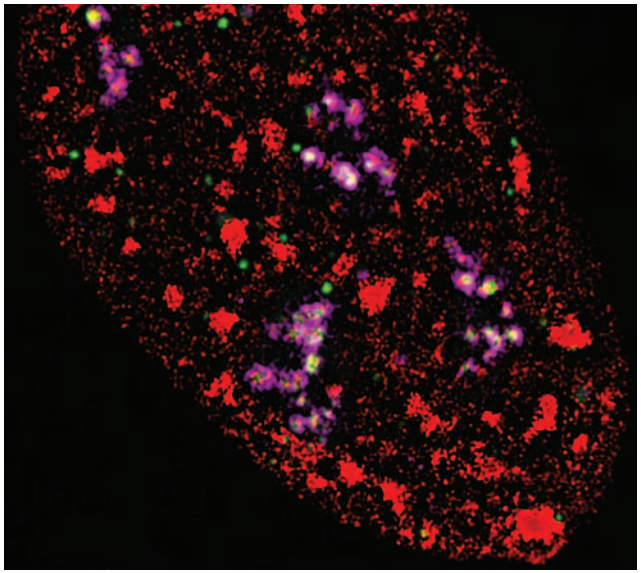

Fig. 1. Super-resolution image of nuclear interior documents yet undiscovered relationships of nuclear components (green: nucleolar protein UBF, red: phospholipid PIP2, magenta: nucleolar protein fibrillarin)

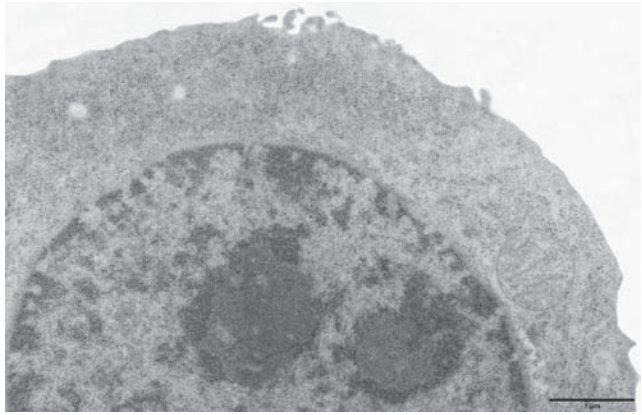

Fig. 2. Combinations of novel sample preparation techniques allows visualization of cells in electron microscopy even without chemical fixation. This human cancer cell was prepared using high-pressure freezing, freeze substitution and embedding in LR White resin.

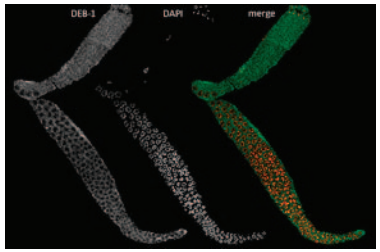

Fig. 3. Actin-binding protein vinculin might play an important role in gametogenesis: immunofluorescence localization of vinculin homologue DEB-1 in a gonad of C. elegans.

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From the left:
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Not in the picture:
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Laboratory of Cell and Developmental Biology

Colorectal cancer, Hypermethylated in Cancer 1, intestinal stem cells, TCF/LEF transcription factors, Wnt signalling

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The single-layer epithelia of the small intestine and colon represent the most rapidly self-renewing adult tissue that completely regenerates approximately every five days. The long-lived stem cells located at the bottom positions of microscopic invaginations called crypts feed an upward compartment of transit-amplifying cells. On migrating up, cells terminally differentiate towards secretory goblet and enteroendocrine cells or absorptive enterocytes. Paneth cells of the small intestine are the only exception to this scheme. These cells produce antibacterial agents and stay at the crypt base, where they persist for three to six weeks.

The proper maintenance of epithelial architecture is controlled by various signalling pathways that regulate the balance between the opposing processes of proliferation and differentiation. Importantly, the majority of these pathways are deregulated in cancer.

The scientific goal of the laboratory is to characterize the molecular mechanisms driving the fate of healthy or diseased intestinal epithelial cells. Since the so-called Wnt pathway is the principal signalling network regulating behaviour of intestinal stem cells, our main focus is to identify genes activated by the Wnt pathway and/or encoding proteins directly involved in the intracellular signal transduction cascade. An important result in recent years was the identification of Troy as a novel modulator of Wnt signalling in the population of fast-cycling stem cells of the small intestine. Particular types of intestinal cancer, both sporadic and hereditary, can be recapitulated in genetically engineered mice. Therefore, the laboratory is using the gene-targeting technology in mouse embryonic stem cells to produce mouse strains that can bring new insights about the signalling mechanisms functioning in the gut tissue. Furthermore, we generated several "reporter" mice allowing lineage tracing experiments in mouse embryonic and adult tissues.

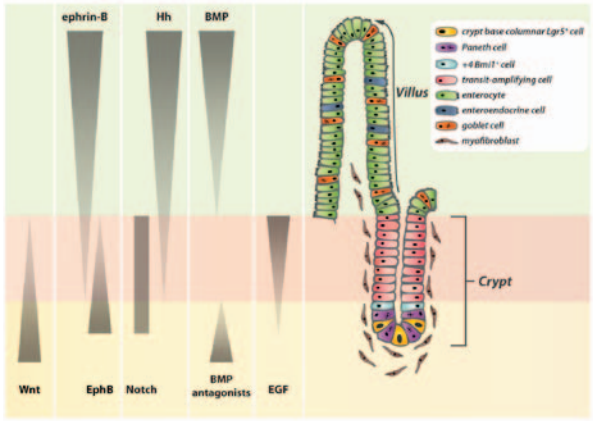

Fig. 1. Architecture of the small intestine epithelium and pathways governing its fate. The proper homeostasis of the intestinal epithelium is regulated by an interconnected network of principal signalling pathways that govern the balance between proliferation and lineage specification.

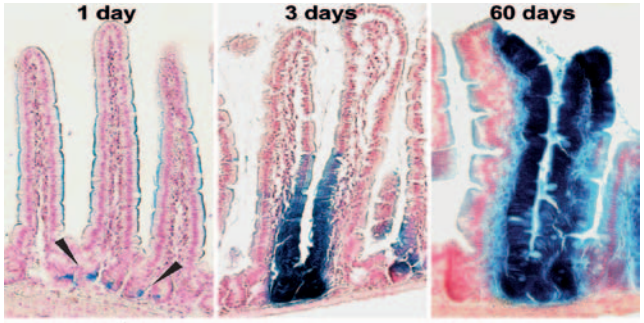

Fig. 2. Lineage tracing in the intestinal epithelium of the Troy-CreERT2 transgenic mice crossed with Rosa26-STOP-lacZ reporter mice. Histochemical detection of the LacZ activity in the duodenum 1 day, 3 days and 60 days after tamoxifen administration. One-day induction generates LacZ-positive cells located at the crypt base (black arrowheads). At later time points, "blue" cell clones were moving upwards from the crypt and reached the top of the villus.

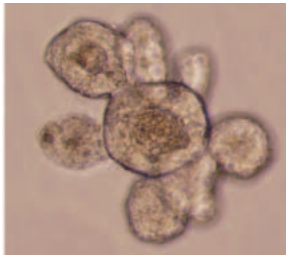

Fig. 3. Stereomicroscopic images of the organoid established from the intestinal crypt.

- 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
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- GA CR, GAP305/11/1780 - Wnt signalling in self-renewal and tumorigenesis of the intestinal epithelia, 2011-2014, V. Kořínek
- Ministry of Industry and Trade, FR-TI4/802 - Development of new chemical compounds with anti-tumour activities or use in regenerative medicine, 2012-2014, V. Kořínek (Co-investigator)
- GA CR, GAP305/12/2347 Molecular mechanisms of the tumour suppressor function of the HIC1 gene, 2012-2015, V. Kořínek
- Operational Programme 'Education for Competitiveness', Founding the Centre of Transgenic Technologies, 2012-2015, V. Kořínek (Guarantor)

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From the left:
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Laboratory of Transcriptional Regulation

Development and evolution, Pax genes, Wnt/β-catenin signalling, Tcf/Lef

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We are interested in development and evolution of development (evo-devo). Our focus is on the role of transcription factors and signalling cascades, especially on the role of the Wnt/β-catenin signalling pathway and transcription factors of Pax and Tcf/Lef families. A combination of gain-of-function (transgenic) and loss-of-function (conditional knock-outs) approaches is used to study mammalian development using laboratory mouse as a model organism. In addition, cell culture approaches are used to study the role of the Tcf/Lef family during induced pluripotency. Our second main interest in the Laboratory is evolution of animal development. Several model systems including amphioxus, platynereis, fish and cnidarians are used in the laboratory to study various aspects of evo-devo.

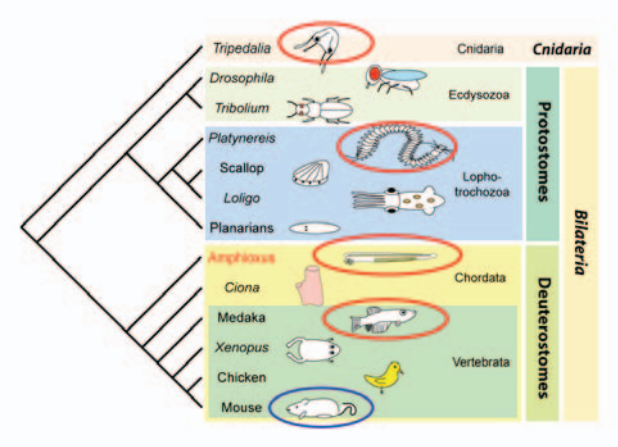

Fig. 1. Main model organisms used in the Laboratory of Transcriptional Regulation.

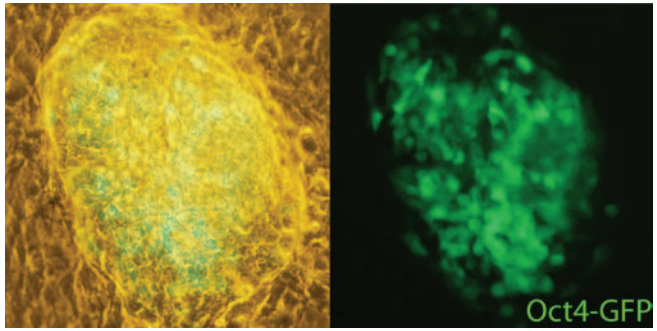

Fig. 2. Induction of pluripotent cells using PiggyBac-OSKM transposon. Activation of endogenous Oct4 is visualized by Oct4-GFP reporter.

- GA CR, GD204/09/H058 - Intercellular signalling in development and disease, 2009-2012, Z. Kozmik
- AS CR, IAA500520908 - The role of Pax genes in eye evolution, 2009-2013, Z. Kozmik
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- Ministry of Education, Youth and Sports of the Czech Republic, LK11214 LK-NÁVRAT - Genetic regulation of embryonic development of the brain and the eye, 2012-2016, O. Machoň
- Ministry of Education, Youth and Sports of the Czech Republic, LH12047 LH-KONTAKT II - The role of alternative splicing in evolution of vertebrate body plan, 2012-2015, Z. Kozmik
- GA CR, GAP305/12/2042 – The role of transcription factors Tcf in the induced pluripotency and during neurogenesis , 2012-2015, O. Machoň

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4. Waaler J, Machon O, Tumova L, Dinh H, Korinek V, Wilson SR, Paulsen JE, Pedersen NM, Eide TJ, Machonova O, Gradl D, Voronkov A, von Kries JP, Krauss S. A novel tankyrase inhibitor decreases canonical Wnt signaling in colon carcinoma cells and reduces tumor growth in conditional APC mutant mice. **Cancer Res 2012** 72(11): 2822-2832.
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Not in the picture:
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Laboratory of Molecular and Cellular Immunology

Genetics of pathogenesis of leishmaniasis, gene mapping, functional diversity, general and species-specific control

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The research programme of the laboratory aims to identify genes and molecular mechanisms involved in the 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. 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 the mouse. Our approach uses a combination of genetic dissection with screening of a large set of immunological and clinical parameters of the disease. We detected 21 *Lmr* (*Leishmania major* response) genes and mapped them to distinct chromosomal loci and we found that gene effects on the 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 response, and pathological symptoms are regulated independently. Thus, these studies revealed a network-like complexity of the combined effects of the multiple functionally diverse QTLs (quantitative trait loci). Recently, we established the first genetic model of susceptibility to *L. tropica*, a species that similarly as *L. major* causes cutaneous leishmaniasis in humans, but can also visceralize and cause systemic illness. Comparison of the response to *L. major* and *L. tropica* in mouse strains revealed clearly different patterns in the strains' susceptibility to *L. tropica* and *L. major*, which demonstrates existence of species-specific controlling host genes with different functions. This information provides the first step to distinguishing the species-specific from the general genes controlling pathogenesis of leishmaniasis. Therefore, without combining the two components of variation involved in the outcome of *Leishmania* infection – genetic variation of the host and species of the parasite – the understanding of the mechanisms of disease will remain incomplete.

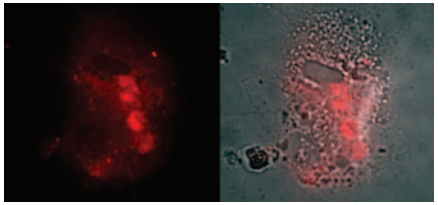

Fig. 1. ROX (Reactive Oxygen) expression in the macrophage after LPS stimulation and *L. major* infection detected with CellROX® Deep Reagent.

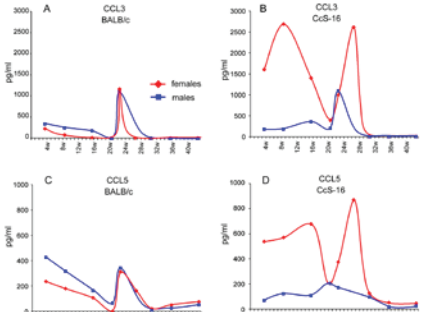

Fig. 2. Unique systemic chemokine response of females of strain CcS-16 after infection with *Leishmania tropica*.

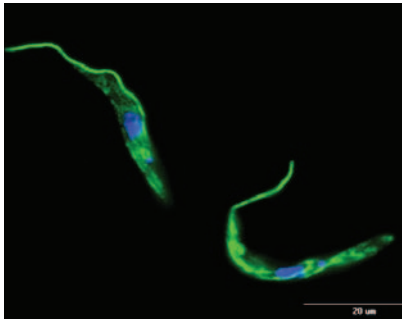

Fig. 3. *Trypanosoma brucei brucei*.

- 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
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Martina Slapničková, MSc / Research Assistant · Jarmila Vojtíšková, PhD / Research Fellow · Yahya Sohrabi, MSc / PhD Student · Valeriya Volkova, MSc / PhD Student · Marie Lipoldová, Assoc Prof, PhD / Head of Laboratory · Matyáš Šíma, MSc / PhD Student · Tetyana Kobets, PhD / Postdoctoral Fellow · Igor Grekov, PhD / Postdoctoral Fellow · Monika Buddeusová / Technician

Not in the picture:
Elena Gusareva, PhD / Postdoctoral Fellow (currently abroad) · Iva Kolářová (Rohoušová), PhD / Postdoctoral Fellow (part time) · Helena Havelková / Research Assistant (until March 2012) · Marie Čepičková, MSc / PhD Student (maternity leave) · Iryna Kurey, MSc / PhD Student

Laboratory of Tumour Immunology

Anti-tumour immunotherapy, immunoediting, immunoepigenetics

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The capacity of tumour cell populations to escape from antitumour immunity in the course of tumour development represents a serious obstacle to the development of effective anti-tumour immunotherapy or vaccination. In our laboratory, we are mainly focused on selected reversible processes, such MHC class I deficiency or altered expression of co-stimulatory/inhibitory molecules, by which tumour cells can escape from specific immune responses. In last several years, we have been interested in epigenetic mechanisms underlying reversible MHC class I downregulation on tumour cells, as well as in the design of immunotherapy/vaccination that would be effective against MHC class I-deficient tumours. Using murine models for MHC class I-deficient tumours (e.g. cervical carcinoma, prostate cancer), in which the MHC class I expression could be restored by cytokines, we have documented association of the MHC class I cell surface expression and DNA methylation of the regulatory regions of the antigen-presenting machinery genes. We have also found that DNA methyltransferase inhibitors induced expression of the 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 inhibitory molecules. In vivo experiments documented the efficacy of immunotherapy of MHC class I-deficient tumours combined with administration of 5-azacytidine, a DNA methyltransferase inhibitor. Our results also suggest an important role of the DNA methylation in the interferon γ-induced expression of antigen-presenting machinery genes. 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. Besides DNA methyltransferase inhibitors, we have investigated other immunomodulatory chemotherapeutics, such as cyclophosphamide, especially in terms of the impacts of immunoactive molecule expression on tumour cells. Further, our areas of interest are populations of immunoregulatory cells and their dynamics and function in the course of chemotherapy. Recently, we have characterized in detail the immunosuppressive character of myeloid-derived suppressor cells induced by cyclophosphamide administration in mice.
In our projects we have been interested in experimental anti-tumour immunotherapy and vaccines, with a special attention paid to the minimal residual tumour disease treatment. 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.

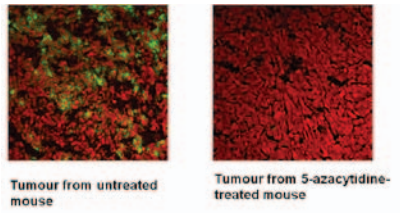

Fig. 1. Fluorescence staining of Gr-1-positive tumour infiltrating cells in tumours. Sections from large (cca 1.5 cm diameter) tumours derived from untreated and 5-azacytidine-treated (i.p. one 200 mg dose) were stained for the presence of Gr-1 marker-positive cells. Most of these cells represent myeloid-derived suppressor cells. Their number in the tumour microenvironment upon the treatment of mice with 5-azacytidine decreased.

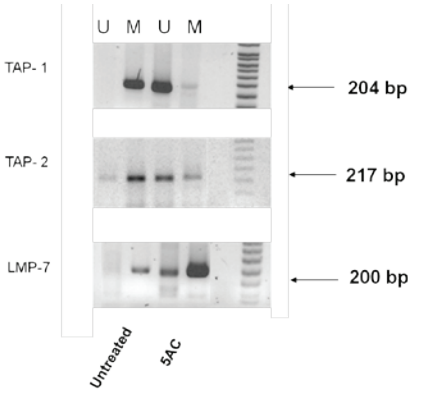

Fig. 2. Methylation specific PCR (MSP) analysis of selected antigen presenting genes promoter sequences in tumour cells from mice treated with 5-azacytidine. DNA from the TC-1/A9 explanted tumour cells from untreated and 5AC-treated animals was bisulphite treated and subjected to the MSP analysis. The methylation status of the representative antigen-presenting machinery genes *TAP-1*, *TAP-2* and *LMP-7* promoter sequences was analyzed. Bands in the lanes designated U represent the PCR products amplified from unmethylated DNA, bands from the M lanes represent the PCR products from methylated DNA.

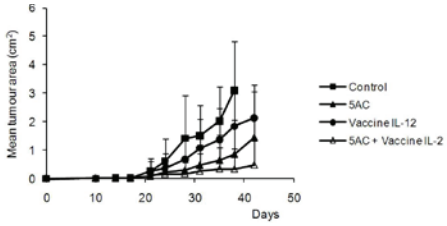

Fig. 3. Combined therapy of murine MHC class I-deficient TC-1/A9 tumours with IL-12-producing vaccine (irradiated TC-1/IL-12 cell line) and 5-azacytidine. Tumour growth curves in control mice and mice subjected to chemo- and immunotherapy and their combination are presented. TC-1/A9 tumour cells were transplanted into syngeneic mice on day 0 and 5-azacytidine (5AC) was repeatedly administered on days 3, 7, 10, 14, 17, 21, 24 and 28, IL-12-producing cells (Vaccine IL-12) were administered on day 4. Significant inhibition of the tumour growth was observed in all treated mice, as compared to the untreated controls. Moreover, combined chemoimmunotherapy was more effective, as compared to monotherapies only.

- EU FP, Network of Excellence, 018933 - Clinigene, European Clinical Gene Transfer Advisory Network, 2006-2011, J. Bubeník
- GA CR, GA301/07/1410 - Immunosuppressive cell populations in the course of tumour progression and therapy, 2007-2011, M. Reiniš
- Ministry of Health of the Czech Republic, NS10660/2009 - Development of experimental anti-WT 1 vaccine for immunotherapy of tumours, 2009-2011, M. Indrová (co-investigator)
- 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
- GA CR, GPP301/11/P220 - Mechanisms underlying cyclophosphamide-induced accumulation of myeloid derived suppressor cells, 2011-2013, R. Mikyšková

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Not in the picture:
Veronika Hrušková / Diploma Student · Anna Žlabová / Diploma Student (until 2012) · Prof Jan Bubeník, MD, DSc (until 2011)

Laboratory of Structural Biology

Protein crystallography, HIV protease, antibody engineering

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The main interests of our group are structural studies of various proteins of biological or medicinal interest using protein crystallography. We use the structural knowledge in understanding the protein function and in some projects also in modulating its function by design of specific inhibitors. Among our targets, there are enzymes from pathogenic organisms (1), as well as human enzymes (4, 5).

Our group also focuses on engineering recombinant antibody fragments of potential diagnostic use (e.g. against carbonic anhydrase IX, a cancer marker). We employ several approaches aiming at practical use of recombinant antibody fragments. For instance, we recently constructed single-chain variable fragment, scFv, comprising an auxiliary polypeptide segment which is rich in tyrosine. This protein shows a higher capacity to bind iodine radionuclide, as compared to the parental scFv (2). We also participated in design of targeted polymers carrying toxic payloads or fluorescence tags (3). The contact of the scFv with the polymer is mediated by the interaction of two peptides forming coiled-coil interface. Such interaction is specific and does not require any other chemistry for antibody-polymer conjugation.

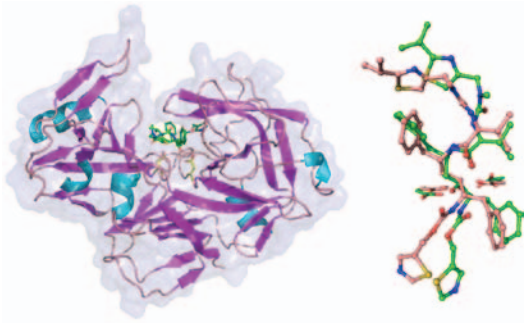

Fig. 1. Crystal structure of secreted aspartic protease 1 from Candida parapsilosis (Sapp1p) in complex with anti-HIV drug ritonavir (see reference 1 for details). The protein in the ribbon representation is coloured by secondary structure, the drug molecule is represented by sticks. Right panel shows comparison of ritonavir binding modes in the active sites of Candida protease Sapp1p (sticks coloured green) and HIV protease (sticks coloured pink).

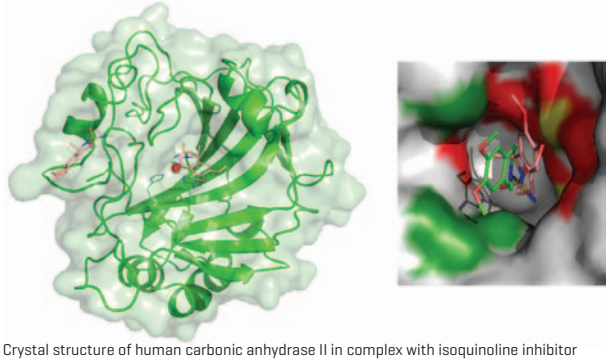

Fig. 2. Crystal structure of human carbonic anhydrase II in complex with isoquinoline inhibitor (see reference 4 for details). The main chain of the protein is represented by a ribbon and a transparent solvent accessible surface. The zinc ion is shown as a red sphere with three coordinating histidine residues in sticks. Inhibitor is depicted in the stick model with carbon atoms coloured pink. Right panel shows unusual inhibitor binding mode (compare with similar compound in green in canonical binding mode) providing clues for the future design of selective inhibitors.

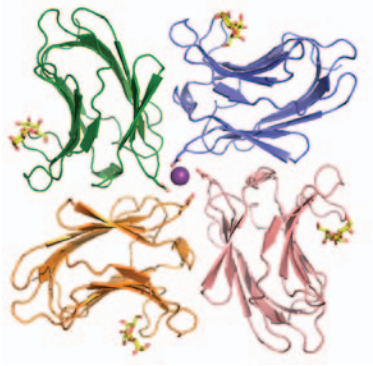

Fig. 3. Crystal structure of mouse galectin-4 in complex with lactose (see reference 5 for details). Crystallographic tetramer is shown with individual monomers in cartoon representation and lactose bound to the carbohydrate binding site as sticks.

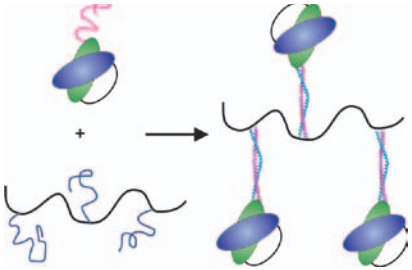

Fig. 4. Schematic representation of targeted polymers (see reference 3). The contact of the antibody fragment with the polymer is mediated by the interaction of two peptides forming specific coiled-coil interface.

- Ministry of Education, Youth and Sports of the Czech Republic, 1M0505 - Centre of targeted therapeutic drugs, 2005-2011, M. Fábry
- Ministry of Industry and Trade, 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
- TA CR, TA02010797 - Labeling of recombinant antibody fragments with use of microfluidic systems, 2012-2014, J. Sedláček

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From the left:
Věra Mrkvičková / Technician · Jitka Kredbová / Technician · Juraj Sedláček, DSc / Research Fellow · Petr Pachl, MSc / PhD Student · Milan Fábry, PhD / Research Fellow · Pavlína Maloy Řezáčová, PhD / Head of Laboratory · Jana Písačková, MSc/ PhD Student · Vlastimil Král, PhD / Research Associate · Irena Sieglová, MSc / Research Assistant

Not in the picture:
Jiří Brynda, Assoc Prof, PhD / Research Fellow · Pavel Mader, PhD / Postdoctoral Fellow · Veronika Krejčiříková, PhD / Postdoctoral Fellow [maternity leave] · Magdalena Hořejší, MSc / Research Assistant · Petr Těšina, MSc / PhD Student

Laboratory of Transgenic Models of Diseases

Proteases and their inhibitors, transgenesis, embryogenesis, aging and epigenetics, neural development

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Our department has an exceptional role in IMG, serving as an incubator in which new research projects and groups as well as research infrastructure (partly core facilities) develop for the project of BIOCEV. Although thematically distinct, all groups and projects are based on the usage of mouse models as a tool to reveal gene functions in the complexity of the whole organism.
Proteases in physiology and disease. One part of the department is focused on proteases, particularly on matrix metalloproteinases (MMP), a disintegrin and metalloproteinase (ADAM), and kallikreins (Klk). While MMP and Klk proteases are largely responsible for controlling extracellular matrix-cell interactions affecting cell differentiation, survival, migration, and other processes, the ADAM proteinases such as ADAM 10 and ADAM17 (TACE) release ligands and their receptors from the cell surface, thus guiding bioavailability of many important regulatory molecules. The balance among the proteases and their natural inhibitors determines whether tissues and organ architecture are to be built up or disrupted, or whether biological processes are to be initiated or terminated. This balance is pivotal for tissue homeostasis and its disturbance may lead to development of various pathologies.
Stem cell pluripotency and early embryonic development. Stochastic processes underlie much of early pre-implantation development but later, especially during gastrulation, increasingly deterministic signalling restricts the developmental fate. Using unique mouse models and environmental stressors we address the molecular mechanisms influencing cell fate decisions probabilistically and the effects this has on embryonic development, stem cell pluripotency, and embryonic robustness to environmental stressors and teratogens.
Stem cell dynamics and aging. In building a quantitative model of epigenetic silencing, we have uncovered an important role for probability-based events. Using several novel mouse mutants found in an unbiased forward genetics screen to alter these odds (including Foxo3a, which has already been linked to human longevity) we are gaining new understanding about how probabilistic cellular events underlie many aspects of the aging process.
Molecular mechanisms of neural development and neurodegeneration. Tight regulation of signalling cascades and molecular mechanisms controlling neuron polarization and axon guidance are essential for normal neural development and function of the nervous system. Their defects or aberrant activation in adult neurons are associated with onset of neurodegenerative disorders, e.g. Alzheimer's disease. Using in vitro systems and in vivo mouse models, we focus on the regulation of signalling cascades by conformational changes, and their role in the nervous system development, aging and disease.

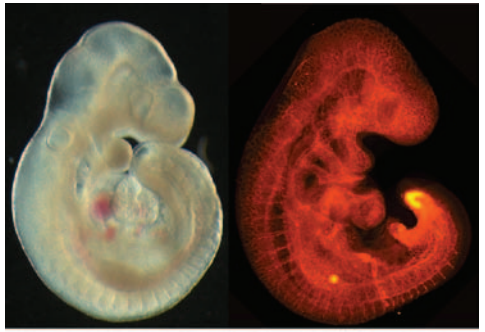

Fig. 1. 9.5 days post coitus mouse embryo; left panel: fresh preparation, right panel: fixed and stained with VE-cadherin antibody to visualized developing vasculature

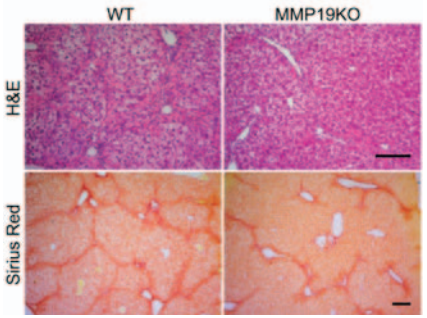

Fig. 2. 6 week CCl4 treatment results in lower liver damage in MMP19KOs compare to WTs. H&E staining showed larger bridging necrosis areas in WTs than in MMP19KOs. Scale bars=200 μm.

- GA AS CR, IAA500520812 - Investigating the pathologic role of metalloproteinases in liver using transgenic mice with conditional expression of transgenes, 2008-2012, R. Sedláček
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- Ministry of Education, YOuth and Sports of the Czech Republic, LK11213 - Analysis of genes Pin1 and CRMP2A and their impact on axon growth, branching and regeneration in vitro and in vivo, 2012-2016, M. Balaštík
- Ministry of Education, YOuth and Sports of the Czech Republic, LM2011032 - INFRAFRONTIER-CZ- Czech Centre for Phenogenomics as a national centre of "The European infrastructure for phenotyping and archiving of model mammalian genomes": Integration of the Czech national centre into international network, 2012-2015, R. Sedláček
- Operational Programme 'Education for Competitiveness', CZ.1.07/2.3.00/30.0027 - Founding the Centre of Transgenic Technologies, 2012-2015, R. Sedláček

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From the left:
Attila Juhász / Technician · Ivan Aleksandrov Kanchev, PhD / Research Fellow (since 2012) · Kateřina Jeřábková, MSc / PhD Student (since 2012) · Kateřina Cibulková, MSc / PhD Student (since 2012) · Monika Červinková, MD, PhD / Research Associate (since 2012) · Martin Balaštík, PhD / Group Leader (since 2012) · Sandra Potyšová, MSc / Research Assistant · Veronika Grešáková, MSc / PhD Student (since 2012) · Sajit Thottathil Oommen, PhD / Postdoctoral Fellow (since 2012) · Trevor Allan Epp, PhD / Group Leader (since 2012) · Karel Chalupský, PhD / Research Fellow · Shohag Bhattacharyya, MSc / PhD Student (since 2012) · Martin Gregor, PhD / Group Leader · Libor Daněk, MA / Project Manager · Kallayanee Chawengsaksophak, PhD / Group Leader (since 2012) · Jana Šafránková, BA / Project Manager (since2012) · Slavomír Kinský, PhD / Postdoctoral Fellow (since 2012) · Lenka Sarnová, MSc / PhD Student · Assoc Prof Radislav Sedláček, PhD / Head of Laboratory · Petr Kašpárek, MSc / PhD Student · Björn Schuster, PhD / Research Associate (since 2012) · Markéta Picková / Technician (since 2012) · Irena Barnetová, PhD / Postdoctoral Fellow (since 2012) · Veronika Libová, MSc / Research Assistant · Henrieta Pálešová, MSc / Technician · Olga Žbodáková, MSc / Research Assistant

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Markéta Jiroušková, PhD / Research Fellow (maternity leave) · Agnieszka Kubik-Zahorodna, PhD / Research Associate (since 2012) · Jolana Turečková, PhD / Research Associate · Dana Průková, PhD / Postdoctoral Fellow · Gizela Koubková, PhD / Research Assistant (since 2012) · Eliška Machalová / Technician (maternity leave) · Václav Gergelits / Diploma Student · Halka Buryová / Diploma Student (until 2012)

Laboratory of RNA Biology

pre-mRNA splicing, spliceosome, epigenetics, retinitis pigmentosa

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Our long-term interest is to determine how cells decode information stored in the genome. We focus on molecules called RNAs that serve as messengers between DNA and proteins. Information in RNAs is fragmented and we analyse how the different fragments of RNA are recognized and joined together. This process is called RNA splicing and we mainly focus on splicing variations among different cells and 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 fascinating processes directly in living cells, we widely employ various microscopy techniques (e.g. fluorescent microscopy, live cell imaging, fluorescence cross-correlation microscopy, and other).

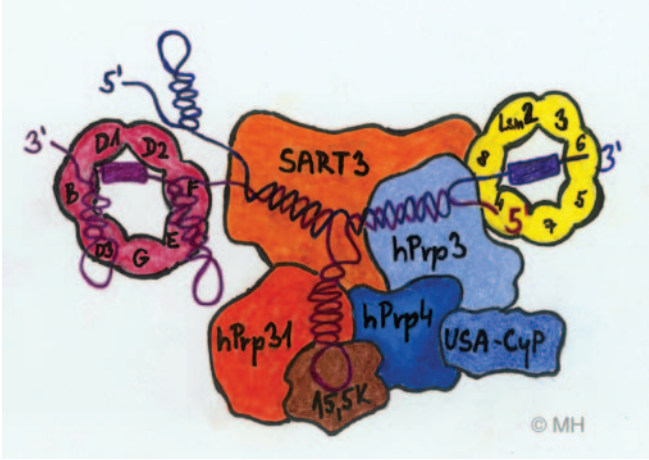

Fig. 1. Schematic representation of a small nuclear ribonucleoprotein particle, a basic building block of the spliceosome (drawing by Martina Huranová).

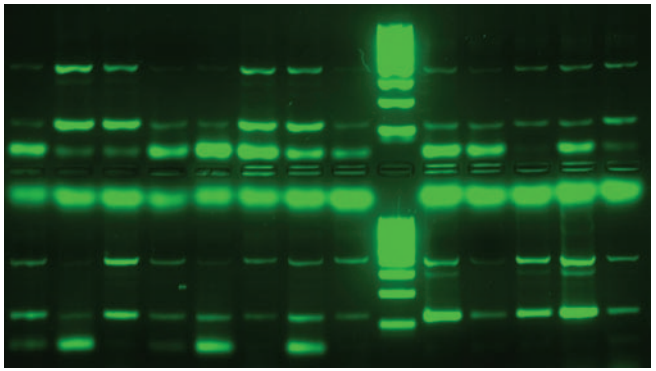

Fig. 2. Analysis of alternative splicing by RT-PCR after inhibition of histone deacetylases (Jarmila Hnilicová).

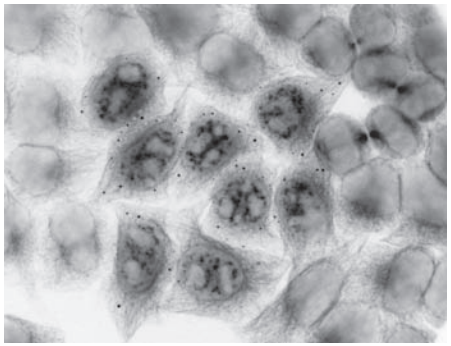

Fig. 3. Human carcinoma cells expressing LSm4 tagged with GFP and stained for microtubules (negative staining by Ivan Novotný).

- 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
- GA CR, GAP302/11/1910 - Formation of splicing machinery in the context of the cell nucleus, 2011-2014, D. Staněk
- GA CR, P305/12/G034 – Centre for RNA Biology, 2012-2018, D. Staněk
- GA CR, GPP301/12/P425 - Functional analysis of hBrr2 mutations linked with retinitis pigmentosa, 2012-2014, Z. Cvačková
- AS ČR, M200521206 – Functional organization of nuclear Cajal bodies with focus on formation of small nuclear ribonucleoprotein particles, 2012-2014, D. Staněk

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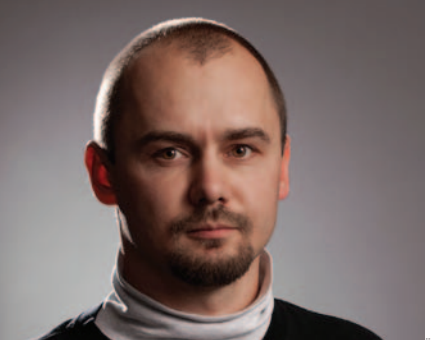
From the left:
Samira Hozeifi, MSc / PhD Student · David Staněk, PhD / Head of Laboratory · Klára Klimešová / Diploma Student · Adriana Roithová / Diploma Student · Ivan Novotný, PhD / Postdoctoral Fellow · Eva Šimková, MSc / PhD Student · Eva Dušková, MSc / PhD Student · Daniel Matějů / Diploma Student · Anna Malinová, MSc / PhD Student

Not in the picture:
Zuzana Cvačková, PhD / Research Associate · Martina Huranová, PhD / Postdoctoral Fellow (until March 2012) · Jarmila Hnilicová, PhD / PhD Student (until December 2011) · Jaroslav Icha / Diploma Student (until June 2012) · Viola Hausnerová / Diploma Student (until June 2011) · Jakub Novák / Diploma Student (until June 2011)



Laboratory of Epigenetic Regulations

RNA degradation, dsRNA, RNAi, miRNA, chromatin

Petr Svoboda

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The zygotic genome activation is the first step in the execution of the genome-encoded programme 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.

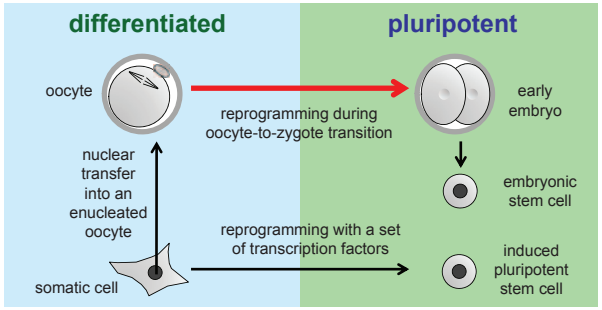

Fig. 1. Oocyte-to-zygote transition is a unique model for studying pluripotency. The mammalian oocyte is a highly specialized cell, whose cytoplasm is capable of reprogramming a genome to initiate development of a new organism. The blastomeres of the 2-cell embryo are totipotent as they can give rise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

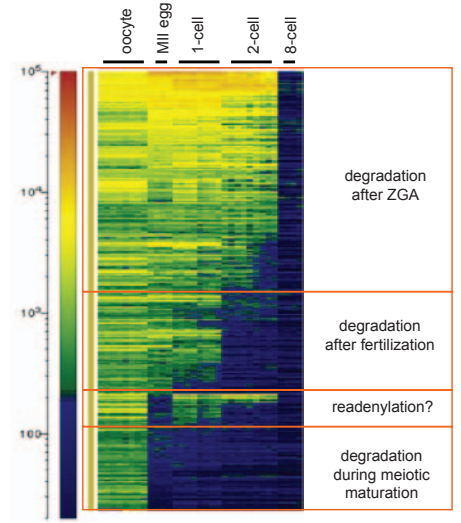

Fig. 2. Microarray analysis of maternal mRNA degradation. Shown are microarray data for 300 maternal transcripts. Colour scale indicates mRNA abundance at different stages.

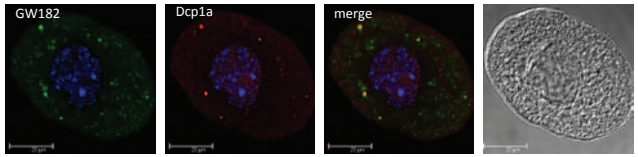

Fig. 3. Co-localization of p-body components GW192 and DCP1A in meiotically incompetent oocytes. P-bodies are centres of mRNA metabolism, including degradation and storage.

- GA CR, GA204/09/0085 - RNA silencing and long dsRNA in mammalian cells, 2009-2013, P. Svoboda
- Ministry of Education, Youth and Sports of the Czech Republic, ME09039 - The role of post-transtranional mechanisms in reprogramming of mouse oocytes into pluripotent cells, 2009-2012, P. Svoboda
- GA CR, GAP305/10/2215 - Control of chromatin and pluripotency by microRNAs, 2010-2013, P. Svoboda
- AS CR, M200521202 – Integrative approach to understanding the mechanism of genome activation and natural occurrence of pluripotency in mammalian embryo, 2012-2015, P. Svoboda

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2. Nejepinska J, Malik R, Moravec M, Svoboda P. Deep sequencing reveals complex spurious transcription from transiently transfected plasmids. **PLoS One 2012** 7(8): e43283.
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4. Nejepinska J, Malik R, Filkowski J, Flemr M, Filipowicz W, Svoboda P. dsRNA expression in the mouse elicits RNAi in oocytes and low adenosine deamination in somatic cells. **Nucleic Acids Res 2012** 40(1): 399-413.
5. Ohrt T, Muetze J, Svoboda P, Schwille P. Intracellular localization and routing of miRNA and RNAi pathway components. **Curr Top Med Chem 2012** 12(2): 79-88.

From the left:
Jana Faltýnková / Diploma Student · Radek Malík, MD, PhD / Research Fellow · Claire Ryan · Petr Svoboda, PhD / Head of Laboratory · Jana Nejepínská, MSc / PhD Student · Radek Jankele / Diploma Student · Kateřina Podolská, MSc / PhD Student · Michaela Vaškovičová/ Diploma Student

Not in the picture:
Kateřina Chalupníková, PhD / Research Associate (maternity leave) · Matyáš Flemr, MSc / PhD Student · Martin Moravec / Diploma Student · Meyer Lansky / Administrative Assistant

Laboratory of Genomics and Bioinformatics

Genome analysis, cancer genomics, next-generation sequencing, transcriptome analysis

Čestmír Vlček

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Genome sequences are the ultimate source for phylogenomics. To understand the evolution of eukaryotes and the developmental processes that they regulate, it is necessary to analyse their genomes and transcriptomes. We sequenced transcriptomes of two multicellular eukaryotes, both of phylum Radiata, sweet water medusa *Craspeducusta sowerbyi* and cubozoan *Tripedalia cystophora*. The expression of selected developmental genes was characterized in situ. Single-cell eukaryotes (protists) 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 main interest is also focused on endosymbiotic origin of two emblematic organelles of the eukaryotic cell, the mitochondrion and the plastid. Using next-generation sequencing platforms we characterize genomes and transcriptomes of many protist species, namely *Diplonema papillatum, Mastigamoeba balamuthi, Andalucia godoyi* and *Malawimonas*. Adding genome sequences from diverse protists to currently available eukaryotic genomes enables us to deduce, with a much higher accuracy, details of many steps and processes of the evolution of the eukaryotic cell. A second major topic of our group is directed towards cancer genomics. Using the Illumina microarray technique, we study intracellular interactions in malignant melanoma and identify markers specific for different cancers. We also analyse the endogenous retroviral elements in human that are associated with cancers and explore the anticancer effects of statin.

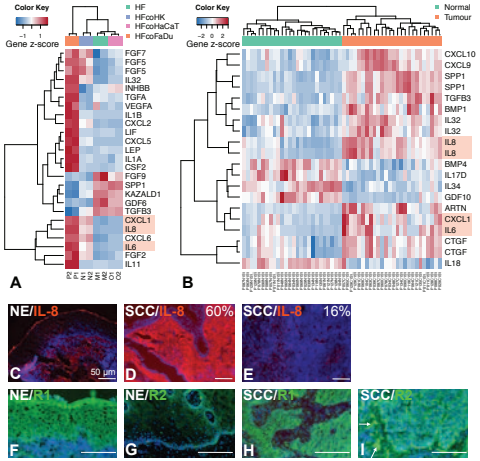

Fig. 1. Expression changes of genes coding for paracrine factors in human fibroblasts cultured with the epithelial cells (A), comparison of tumour and normal tissues of clinical samples of squamous cell carcinoma (B), and expression of selected genes (IL8, CXCL1 and CXCL2) in human tissues visualized by immunofluorescence (C-I).

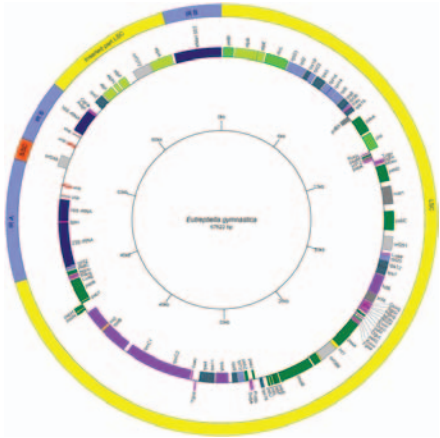

Fig. 2. : Map of the plastid genome of *Eutreptiella gymnastica*.

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

- Ministry of Education, Youth and Sports of the Czech Republic, 1M0520 - Centre for Applied Genomics, 2005-2011, V. Pačes
- Ministry of Education, Youth and Sports of the Czech Republic, 2B06106 - Novel genomic and biotechnological 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
- GA CR, GAP506/11/1317 - Diversity and evolution of anaerobic Heterolobosea, 2011-2014, Č. Vlček
- GA CR, GAP305/11/1061 - Evolution of parasitism: analysis of genomes and key physiological functions of free-living Mastigamoeba balamuthi and pathogenic Entamoeba histolytica, 2011-2015, J. Pačes
- GA CR, GAP506/11/1320 - Establishment of the secondary plastid in euglenids, 2011-2015, Č. Vlček
- Ministry of Health of the Czech Republic, NT13488 - Genomic analysis of tumor-associated fibroblasts in head and neck carcinoma: The basis for new generation of biologic anti-tumor therapy, 2012-2015, H. Strnad
- GA CR, GAP304/12/1333 – Intracellular interactions in malignant melanoma – experimental studies, 2012-2015, H. Strnad
- GA CR, GAP506/12/1010 - Genome sequencing of Oxymonad and Trimastix, 2012-2014, V. Pačes
- Ministry of Health of the Czech Republic, NT13112 - Studies of anticancer effects of statins, 2012-2015, H. Strnad

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4. Melenovský V, Beneš J, Skaroupková P, Sedmera D, Strnad H, Kolář M, Vlček Č, Petrák J, Beneš J, Papoušek F, Oliyarnyk O, Kazdová L, Červenka L. Metabolic characterization of volume overload heart failure due to aorto-caval fistula in rats. **Mol Cell Biochem 2011** 354(1-2): 83-96.
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From the left:
Jan Fousek, PhD / Research Assistant · Miluše Hroudová, PhD / Research Assistant · Čestmír Vlček, PhD / Head of Laboratory · Michal Kolář, PhD / Research Assistant · Jakub Rídl, MSc / PhD Student · Prof Václav Pačes, DSc / Research Fellow · Jan Pačes, PhD / Research Fellow · Šárka Pinkasová / Technician · Hynek Strnad, PhD / Research Fellow

Not in the picture:
Jana Šáchová MSc / PhD Student (maternity leave)

Information Technologies

Petr Divina

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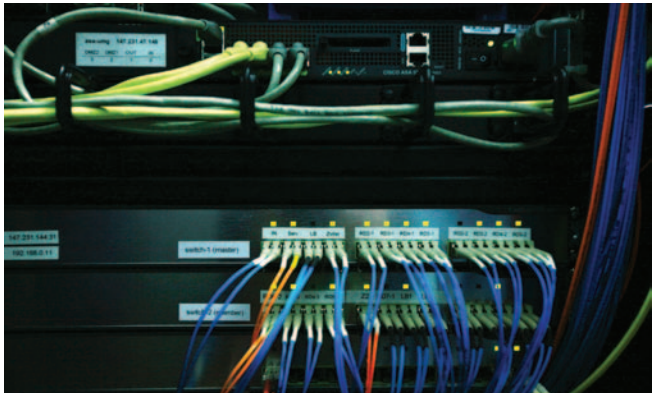
www.img.cas.cz/core-facilities/information-technologies

The IT department provides a wide 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 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.



Firewall and central switch stack

Main data centre room

Computer classroom

Tape library and disk storage

From the left:
Michal Rolník · Juraj Kopčan, MSc (since 2012) · Michal Kůs · Petr Janků, MSc · Petr Divina, PhD / Head

Not in the picture:
Tomáš Drexler (until 2012) · Miroslav Indra, PhD · Jakub Šimon (until 2012)

Genomics and Bioinformatics

Hynek Strnad

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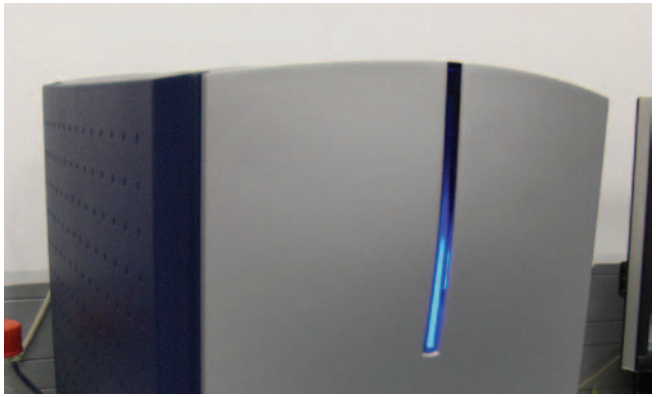
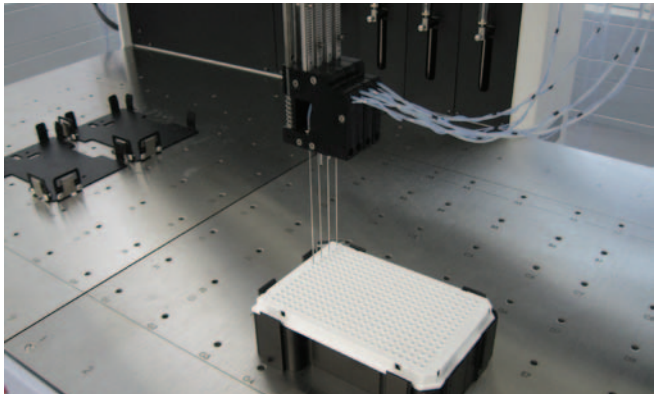
www.img.cas.cz/core-facilities/genomics-and-bioinformatics

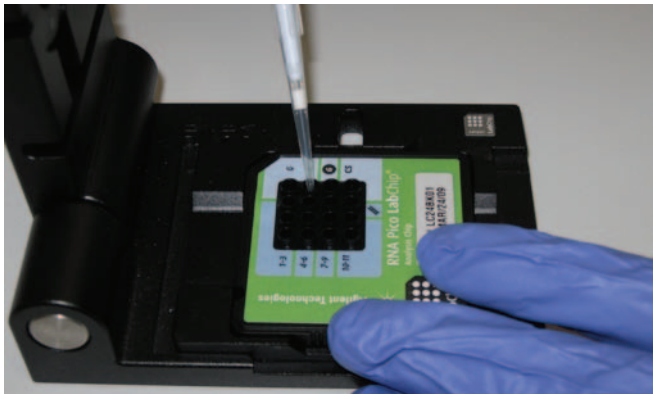
The facility was established in late 2005 after purchase of the Affymetrix GeneChip System and was initially operated by the staff from the Department of Mouse Molecular Genetics. Since January 2007, it has become an independent unit which provides full chip microarray services, real-time quantitative 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 the processed samples (spectrophotometer Nanodrop and capillary electrophoresis Agilent Bioanalyzer 2100).



Affymetrix GeneChip Scanner



PerkinElmer Janus



Agilent Bioanalyzer

From the left:
Hynek Strnad / Head · Šárka Kocourková · Martina Chmelíková

Not in the picture:
Veronika Klatovská · Jitka Dubská

Monoclonal Antibodies and Cryobank

Dobromila Matějková

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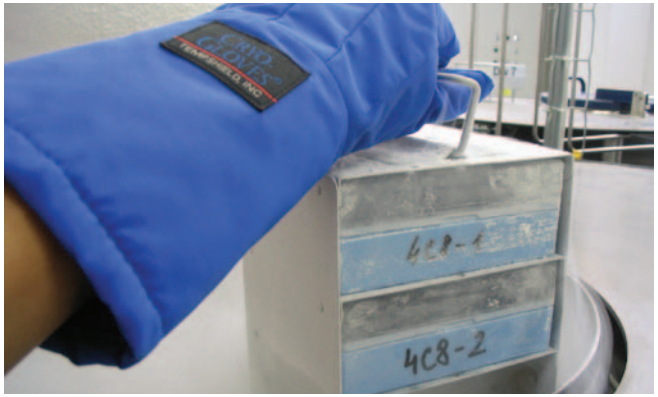
/core-facilities/monoclonal-antibodies-and-cryobank

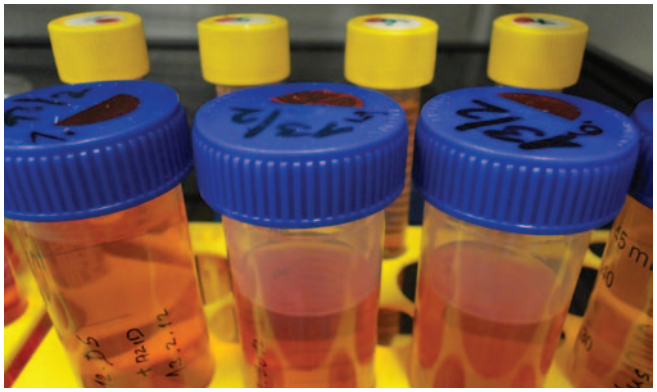
Monoclonal Antibody Facility
The facility provides preparation of mouse monoclonal antibodies including immunization using a particular immunization protocol, ELISA testing of production of specific antibodies, cloning of selected samples, freezing of cryobank samples, cultivation of cell culture supernatants, 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, 02 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.

Cryobank equipment



Cryobank equipment



Cell culture supernatant

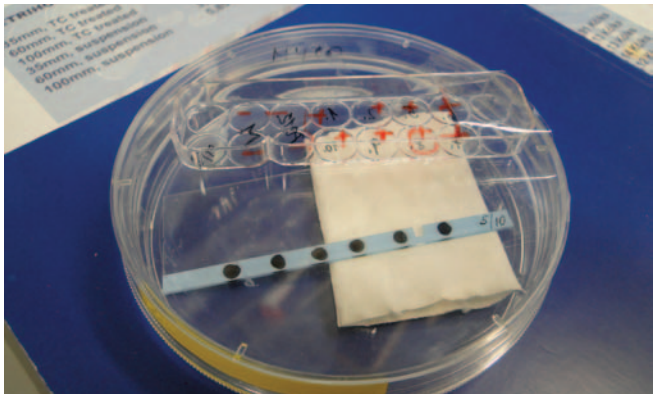


Plate for mycoplasma test

From the left:
Ladislava Sobotková (since 2012) · Hana Korábová · Dobromila Matějková, MSc / Head

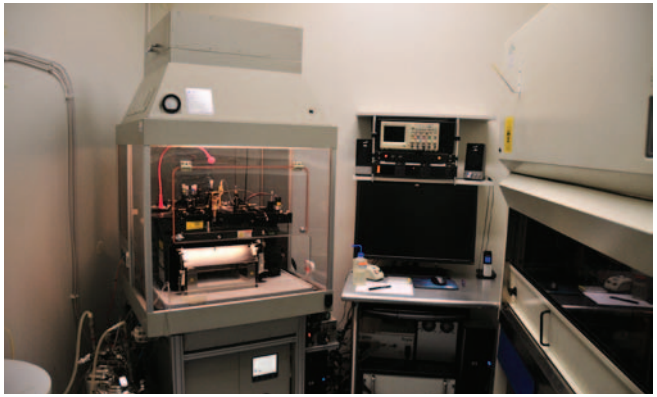
Flow Cytometry and Light Microscopy

Ondřej Horváth

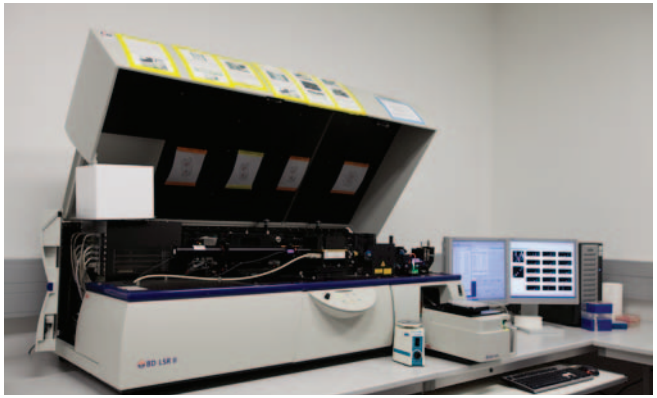
ondrej.horvath@img.cas.cz

www.img.cas.cz/core-facilities/flow-cytometry-and-light-microscopy

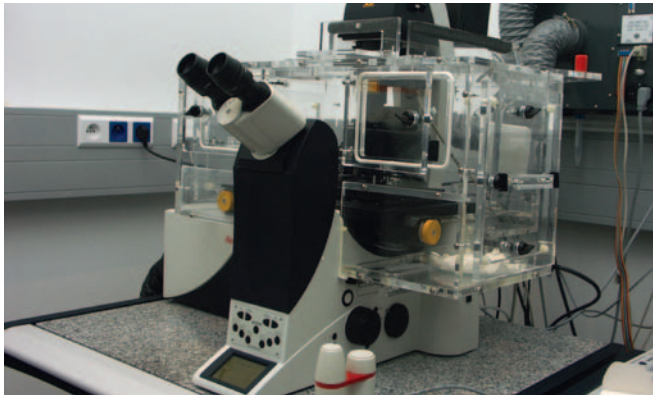
The facility provides methodological and instrumentation background for flow-cytometric and fluorescence microscopy techniques. The facility is equipped with three flow cytometers – two analysers (BD FACSCalibur and BD LSRII) and one sorter. The LSRII instrument is the four-laser (405, 488, 561 and 633-nm) type 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 analysers are equipped with a HTS loader for high-throughput analysis of samples directly from 96- or 384-well plates. Polychromatic high-speed cell sorter BD-Influx is equipped with five lasers (355, 405, 488, 561 and 640nm), 14 fluorescent detectors, small particle option for measuring small particles, cloning deposition unit and 6-way sorting capability. The sorter is located inside the biological safety cabinet and is fully adapted for sterile sorting. 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 fast scanner and three supersensitive HyD detectors (Leica TCS SP5 AOBS TANDEM), Leica inverted fluorescent microscope with TIRF illumination (Leica TIRF MC), and wide-field inverted fluorescence microscope with laser photomanipulation (DeltaVision Core) equipped with InsightSSI solid state illumination light source and UltimateFocus coverslide position maintaining system. All microscopes are equipped with environmental chambers and are suitable for live cell imaging. This state-of-art instrumentation allows the 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, ModFit) and image data (SoftWorx, Imaris, LAS AF, Huygens, Metamorph, ImageJ).



Polychromatic high-speed cell sorter BD Influx



LSRII flow cytometer



SP5 TCS AOBS Tandem confocal microscope

DeltaVision Core deconvolution microscope with laser photomanipulation

From the left:
Zdeněk Cimburek · Ondřej Horváth / Head

Histological laboratory

Vladimír Kořínek

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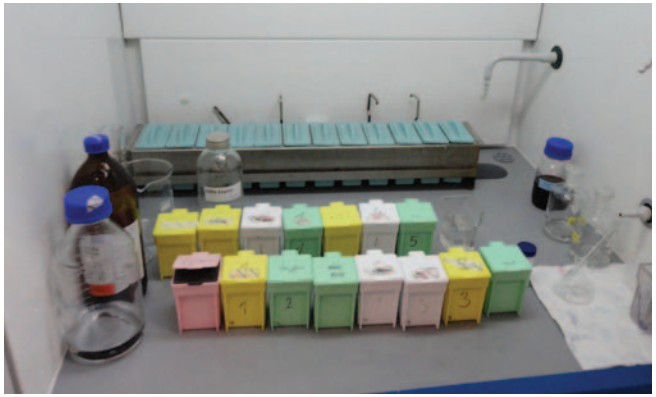
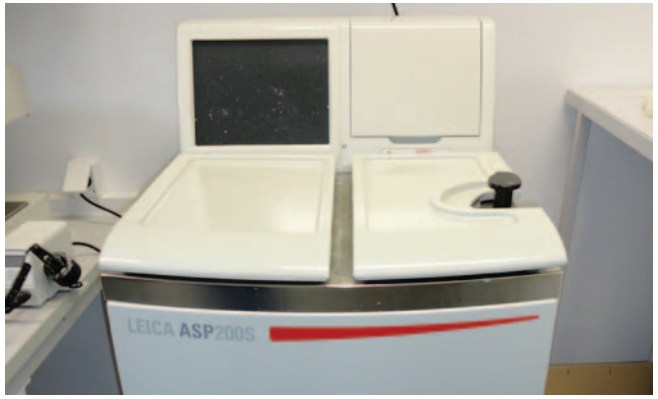
www.img.cas.cz/core-facilities/histology-lab

The laboratory is equipped for tissue dehydration, creation of paraffin blocks, tissue sectioning, deparaffination and antigen retrieval. The facility is based on semi-self-service – tissue dehydration is collective and handled by the staff, and all the other steps are carried out by each user individually. The most important laboratory equipment consists of a set of three Leica devices – tissue processor, paraffin-embedding station and microtome. Tissue processor ASP200S can process up to two hundred samples in standard histological cassettes in a single run. Paraffin-embedding station EG1150H provides full comfort for creation of wax blocks. Fully motorized rotary microtome RM2255 is supplied with various types of blades for easy sectioning of different types of tissues. There is also a set of trays for tissue deparaffination and pressure cooker for antigen retrieval. Since the laboratory has been equipped with financial support of the Academy of Sciences of the Czech Republic, all Academy researchers are welcome to use this facility.



Set of trays for deparaffination



Tissue processor for dehydration

Rotary microtome with equipment

Paraffin embedding station


Leica

Media and Glass Washing

Hana Marxová

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www.img.cas.cz/core-facilities/media-and-glass-washing

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 (vapour 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).

Wash room, loading of a glass washer

Wash room, clean glass coming out of the washer

Wash room, packing and sterilization

Preparation of media, sterilization of solutions

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

Transgenic Unit

Radislav Sedláček

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www.img.cas.cz/core-facilities/transgenic-unit

One of the key challenges in biomedical research is to attribute biological functions to the identified human genes. Although non-mammalian models have delivered valuable knowledge on basic gene functions, the mouse that is genetically very close to humans is ideal to model physiologic functions and diseases of humans and has become a key tool in basic and biomedical research.

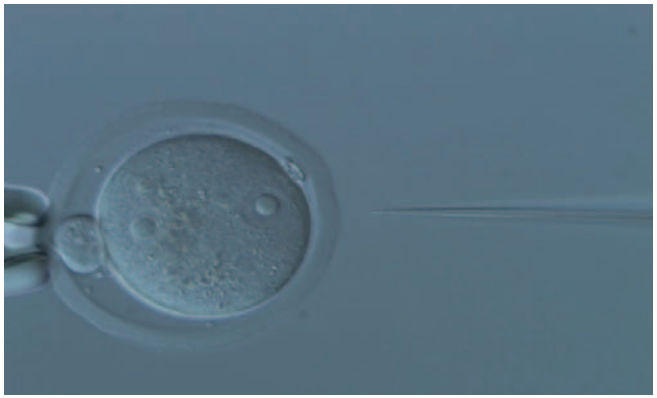
The ability to engineer the mouse genome has indeed transformed biomedical research in the last decade. Transgenic and gene knockout/knockin technologies have become important experimental tools for assigning functions to genes at the level of whole complexity of the organism, creating models of genetic disorders, evaluating effects of potential therapeutic targets and drugs, and thus helping to answer fundamental issues in basic and applied research. It is possible to increase or decrease gene expression, or eliminate the expression of a gene completely, or manipulate with larger genomic areas.

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. Nowadays, TgU also serves international scientific community.

TgU offers pronuclear microinjection of DNA constructs into mouse zygotes for the production of transgenic founders (including zinc-finger and TALE nucleases), microinjection of targeted ES cell lines into blastocysts (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, rederivation of mouse strains and lines, and others (see also our web page at 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 organizations.

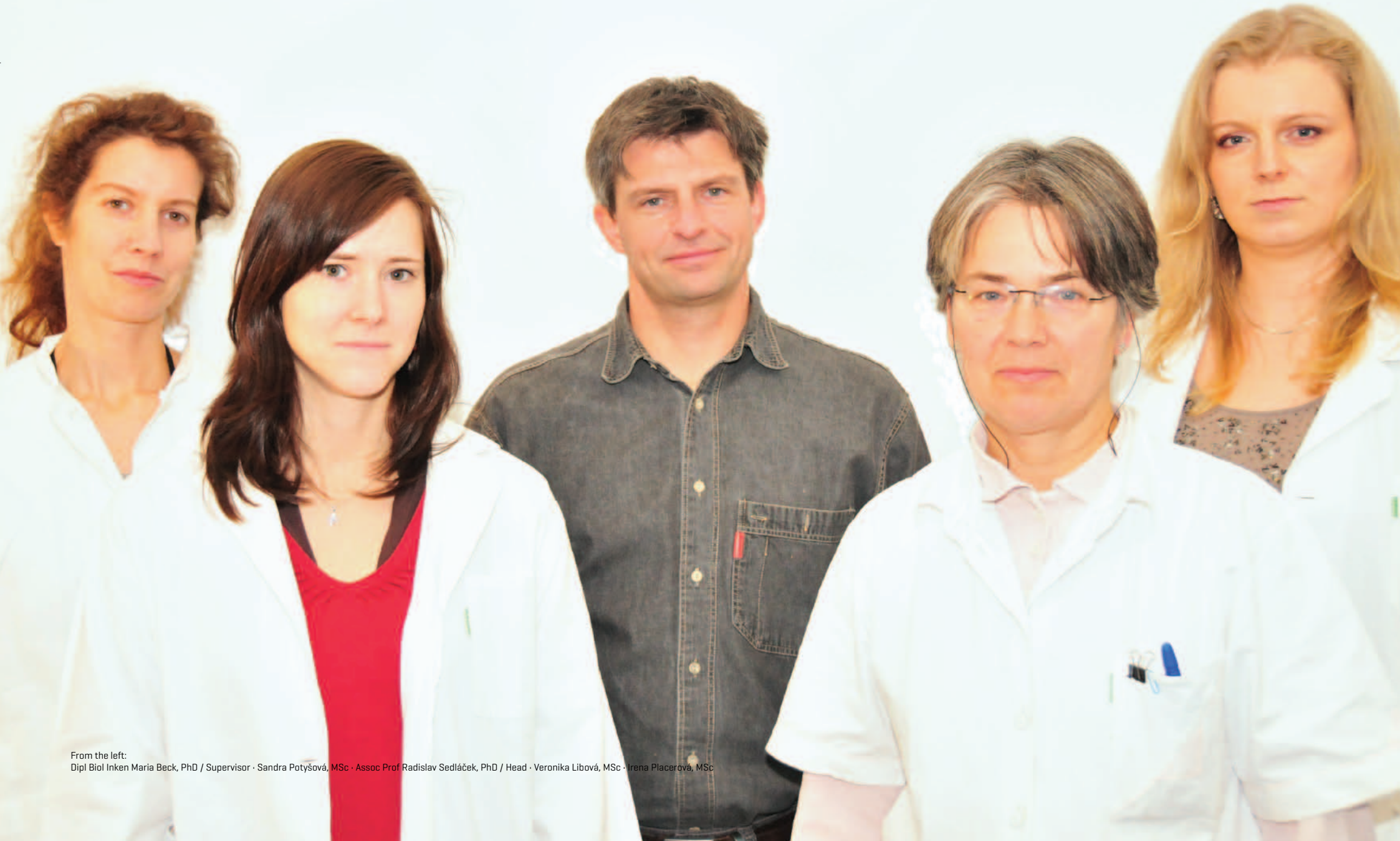
Foster mother with chimeric pups

Implantation of embryos under SPF conditions



Pronuclear injection

Transgenic core facility



From the left:
Dipl Biol Inken Maria Beck, PhD / Supervisor · Sandra Potyšová, MSc · Assoc Prof Radislav Sedláček, PhD / Head · Veronika Libová, MSc · Irena Placerová, MSc

Animal Facility (Mice)

Jan Honetschläger

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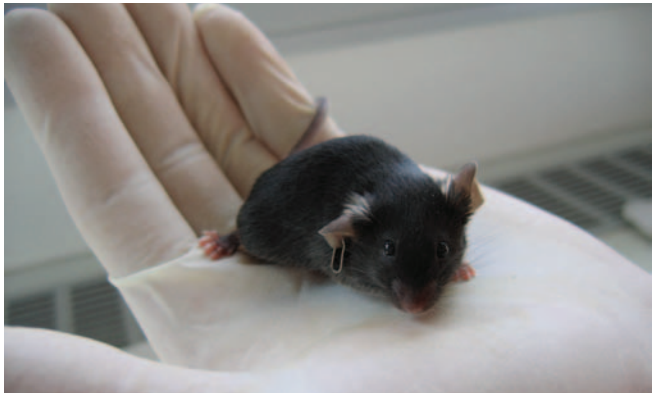
www.img.cas.cz/core-facilities/animal-facility

The IMG animal facility located on the Krč campus is fully accredited for breeding routine animal strains including genetically modified animals. It contains experimental spaces for mice, poultry and fish. The present total capacity is 11,000 cages. Several types of breeding are provided by the facility – conventional breeding, quarantine for imported animals and special barrier SPF breeding operated under the FELASA guidelines. Half of the SPF breeding is performed in individually ventilated cages. The facility is housed in three separate buildings one of which is now under reconstruction. In the near future this building will serve for experimental barrier breeding of mice. One of the buildings houses a therapeutic X-ray instrument enabling regulated irradiation of cells and an instrument for sonography. The animal facility closely collaborates with the Transgenic Unit and is available both to IMG research groups and to scientists from other Academy Institutes.

TGU experimental room

Experimental room with IVC cages



C57 BL/6 mouse

T-200 X-ray

From the left:
Veronika Šobíšková · Emilie Hájková · Kamila Hviščová, Bc · Alena Babanská · Zuzana Nezbedová · Stanislav Dygryn · Daniela Vorlová · Jana Matoušková · Monika Novotná · Jan Honetschläger, DVM, MBA / Head · Jana Březinová · Pavla Kameníková · Kamila Malá · Milan Kudlič · Dagmar Čermáková · Zuzana Bakešová · Miloslava Kudličová · Michaela Lišáková · Romana Kolaciová · Renáta Cihelková

Not in the picture:
Věra Žbánková · Zuzana Novotná, MSc (maternity leave) · Jana Kopkanová, MSc (maternity leave) · Jarmila Krestová (maternity leave) · Lenka Rysslová (maternity leave) · Kateřina Ševčíková (maternity leave)

Animal Facility (Chicken)

Martina Minariková (Ješátková) martina.minarikova@img.cas.cz www.img.cas.cz/core-facilities/animal-facility

This facility is located in the village Koleč, north of Prague, about 45 km from the main campus 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 needed by several research groups focusing on chicken models.

Marking of hatched chicks

Brown Leghorn cocks

The main building

Outbred Leghorn line

Breeding in invidual cages for artificial insemination

From the left:
Eva Bernášková · Alena Eisensteinová · Alena Porazilová · Martina Minariková, MSc / Head · Radomíra Skoková · Petra Faloutová · Jitka Dvořáková · Iva Hrodková · KamilaThunová

Building Maintenance

Miroslav Heyduk

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http://www.img.cas.cz/core-facilities/building-maintenance

Entrance hall

Milan Hašek Auditorium

Central staircase

Foodhall

Meeting point

Cafeteria

From the left:
Jana Boučková · Dana Macková · Daniela Macková · Miroslav Heyduk, MSc / Head · Ivan Dundr · Hana Kozáková · Martina Vilímovská · Eva Wünschová, MSc

Not in the picture:
Jiří Macek, MSc · Petr Blahout

Office of the Director

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From the left:
Jitka Černá (since 2012) · Kateřina Sedláčková · Gabriela Marešová · Prof Jiří Jonák, MD, DSc · Leona Krausová · Šárka Takáčová, MSc / Head

Not in the picture:
Zdeňka Schuhová (until 2011) · Šárka Šímová, PhD (since 2012) · Hana Vágnerová (until 2012)

Finances and Administration

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From the left:
Veronika Takáčová (since 2011) · Milena Petříková · Kateřina Drastilová · Michal Švestka · Renata Schönová / Head · Jitka Třísková (since 2011) · Hana Nezbedová · Jana Immerová (since 2011) · Iva Palacká (since 2012) · Emílie Štorchová · Ivana Brabencová · Klára Knížková (since 2012)

Not in the picture:
Jitka Emanuelová (until 2011) · Jaroslava Samohylová (until 2011) · Miloslava Šnajbergová (until 2012) · Hana Švestková (maternity leave) · Kateřina Sedláčková (since 2012)

BIOCEV Division

Jan Zemánek

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www.img.cas.cz/core-facilities/biocev-division

The BIOCEV division of IMG was established in September 2009. Its main task is to ensure successful implementation of the new research centre project - Biotechnology and Biomedicine Centre of Academy of Sciences and Charles University in Vestec – BIOCEV.

About the project
BIOCEV is a joint project of six institutes of the Academy of Sciences of the Czech Republic and two faculties of Charles University in Prague with its main goal to establish a scientific Centre of Excellence in the two most complex fields of modern science – biotechnology and biomedicine. The purpose of the project is to provide the respected scientists with facilities for innovative research, stimulate the conditions for excellent science work in the Czech Republic and support national as well as European growth of the biotechnology industry.

Project vision
To establish a centre of excellence as part of the European Research Area and to guarantee development of modern biotechnologies and biomedicine in favour of scientific progress and modern society

The project builds upon three pillars of the knowledge triangle:

Scientific programme
The top-quality and internationally competitive scientific programme, focused on the key challenges and latest trends in biotechnology and biomedicine research, is composed of five synergetic fields of biomedicine and biotechnology research: Functional Genomics, Cellular Biology and Virology, Structural Biology and Protein Engineering, Biomaterials and Tissue Engineering and Development of Diagnostic and Therapeutic Procedures. The scientific programme will be supported by several top-quality core facilities, including the Czech Centre for Phenogenomics or the Centre of Imaging Methods.

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.

Centre BIOCEV 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 through the Operational Programme Research and Development for Innovations. The construction should start in the first half of 2013, its operation then in early 2015.

For more information see www.biocev.eu.

BIOCEV

Visualization of the Centre

From the left:
Tomáš Novotný · Eva Andresová · Libor Fabián, MSc · Tomáš Pěnek, MSc · Radka Pinkavová, MSc · Petra Roubíčková · Zbyněk Šmída, PhD · Lucie Kubalošová · Věra Prosová, MSc · Jan Zemánek, MArch / Head · Božena Šléglová · Jan Jirků

Council of the IMG

Vladimír Kořínek, PhD
Chairman

Zbyněk Kozmik, PhD
Vice-Chairman

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 and in the second term of office starting from January 2012, they are:

Petr Bartůněk, PhD
Internal Member

Jiří Forejt, Prof, MD, DSc
Internal Member

Pavel Hozák, Assoc Prof, DSc
Internal Member

Pavlína Řezáčová (Maloy), PhD Internal Member

Radislav Sedláček, Assoc Prof, PhD Internal Member

David Staněk, PhD
Internal Member

External Members

Jan Černý, Assoc Prof, PhD
Faculty of Science, Charles University, Prague

Petr Dvořák, Prof, PhD
Faculty of Medicine, Masaryk University, Brno

Hana Sychrová, DSc
Institute of Physiology of the ASCR, v. v. i.

Tomáš Stopka, Assoc Prof, MD, PhD
First Faculty of Medicine, Charles University, General Faculty Hospital, Prague

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, MBA
Vice-Chairman
Deputy Director, IMG

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

Lucie Kubínová, PhD
Institute of Physiology of the ASCR

David Štůla, BCL
Lawyer

Publications 2011-2012

ww.img.cas.cz/achievements/publications/

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Seminar Speakers 2011

26/01/11	**Lumír Krejčí**	(National Centre for Biomolecular Research and Department of Biology, Masaryk University, Brno)
02/02/11	**Jiří Damborský**	(Loschmidt Laboratories, Department of Experimental Biology, Faculty of Science, Masaryk University, Brno)
01/03/11	**Bernard De Massy**	(Institut de Génétique Humaine, CNRS, Montpellier, France)
09/03/11	**Radek Špíšek**	(Institute of Immunology, Second Faculty of Medicine, Charles University, Prague)
05/04/11	**Antonio R.L. Teixeira**	(Chagas Disease Multidisciplinary Research, Laboratory, Faculty of Medicine, University of Brasília, Brazil)
11/04/11	**Bernhard Herrmann**	(Max Planck Institute for Molecular Genetics, Berlin, Germany)
20/04/11	**Karel Smetana**	(Institute of Anatomy, First Faculty of Medicine, Charles University, Prague)
26/04/11	**Pavel Tomančák**	(Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany)
05/05/11	**Stefan Rose-John**	(Department of Biochemistry, Medical Faculty, Christian Albrechts Universität zu Kiel, Germany)
17/05/11	**Ulrich Hübscher**	(Institute of Veterinary Biochemistry and Molecular Biology, University of Zürich-Irchel, Switzerland)
19/05/11	**Maria Brattsand**	(Department of Public Health and Clinical Medicine, Dermatology and Venereology, Umeå University, Sweden)
27/05/11	**Dalibor Blažek**	(Faculty of Medicine, Masaryk University, CEITEC, Brno)
08/06/11	**Boris Vyskot**	(Institute of Biophysics, Academy of Sciences of the Czech Republic and Masaryk University, Brno)
13/06/11	**Alberto Riva**	(University of Florida, Gainesville, USA)
26/07/11	**Jeffrey Cloutier**	(Division of Stem Cell Biology and Developmental Genetics, Medical Research Council, National Institute for Medical Research, London, UK)
05/09/11	**Lukáš Čermák**	(New York University Medical Center, New York, USA)
07/09/11	**Jiří G. Šafář**	(Departments of Pathology, Neurology, and National Prion Disease Pathology Surveillance Center, Case Western Reserve University, Cleveland, USA)
19/09/11	**Gary Koretzky**	(University of Pennsylvania School of Medicine, Philadelphia, USA)
05 /10/11	**Detlev Arendt**	(Developmental Biology Unit, European Molecular Biology Laboratory, Heidelberg, Germany)
20/10/11	**Jane Wu Feinberg**	(School of Medicine, Northwestern University, Chicago, USA)
21/10/11	**Mark Moore**	(International Mouse Phenotyping Consortium (IMPC))
21/10/11	**Steve Brown**	(IMPC Steering Committee)
21/10/11	**Bernd Kaspers**	(Department of Veterinary Science, Ludwig-Maximilian University of Munich, Germany)
24/10/11	**Daniel Smrž**	(Laboratory of Allergic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, USA)
26/10/11	**Boris Cvek**	(Department of Cell Biology and Genetics, Palacky University, Olomouc)
02/11/11	**Růžena Stránská**	(Centre de Recherche en Cancérologie de Marseille, INSERM, Institut Paoli-Calmettes, Université de la Méditerranée, France)
16/11/11	**Meritxell Alberich-Jorda**	(Harvard Medical School/BIDMC Boston, USA)
16/11/11	**Martin Balaštík**	(Harvard Medical School/BIDMC Boston, USA)
23/11/11	**Robert Černý**	(Faculty of Science, Charles University, Prague)
09/12/11	**Bjoern Schuster**	(Department of Immunobiology, King's College London, UK)

Seminar Speakers 2012

18/01/12	**Jiří Grygar**	(Institute of Physics, Academy of Sciences of the Czech Republic, Prague)
07/03/12	**František Vyskočil**	(Institute of Physiology, Academy of Sciences of the Czech Republic, Prague)
28/03/12	**Jaroslav Flegr**	(Faculty of Science, Charles University, Prague)
11/04/12	**Stanislav Komárek**	(Faculty of Science, Charles University, Prague)
18/04/12	**Ulrich Technau**	(University of Vienna, Austria)
20/04/12	**Mathias Sprinzl**	(Laboratorium für Biochemie, Universität Bayreuth, Germany)
02/05/12	**Stefan Hoppler**	(Institute of Medical Sciences, University of Aberdeen, Scotland)
18/05/12	**Mark Zabel**	(Colorado State University, Fort Collins, USA)
13/06/12	**Ľubomír Tomáška**	(Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia)
15/06/12	**Mark Rogers**	(Sigma Aldrich Field Application Specialist for emerging technologies)
20/06/12	**Bohuslav Dvořák**	(University of Arizona, Department of Pediatrics, Tucson, USA)
26/06/12	**Burkhard Bechinger**	(Chemistry Institute, University of Strasbourg/CNRS, International Centre for Frontier Research in Chemistry, France)
28/06/12	**Larry A. Sklar**	(University of New Mexico, Los Alamos, USA)
20/07/12	**Cristina Maccalli**	(Unit of Immuno-Biotherapy of Melanoma and Solid Tumors, San Raffaele Foundation Scientific Institute, Milan, Italy)
30/08/12	**Nana Sunn**	(Diamantina Institute, University of Queensland, Brisbane, Australia)
05/09/12	**Andrej Luptak**	(University of California, Irvine, USA)
27/09/12	**Tokameh Mahmoudi**	(Erasmus University Medical Centre, Rotterdam, Netherlands)
17/10/12	**Inki Kim**	(Asan Institute for Life Science, Seoul, Korea)
24/10/12	**Héctor Escrivà**	(Groupe Evolution et Developpement des Chordes Observatoire Oceanologique de Banylus sur Mer, France)
25/10/12	**Silke Schumacher**	(European Molecular Biology Laboratory, Heidelberg, Germany)
13/11/12	**Keith M. Skubitz**	(Hematology, Oncology and Transplantation Masonic Cancer Center, University of Minnesota Medical School, USA)
21/11/12	**Christian Lanctôt**	(Institute of Cellular Biology and Pathology, First Faculty of Medicine, Charles University in Prague)

Highlights of 2011-2012

Development of the Institute complex further advanced

In 2011, two additional facilities situated close to the main IMG building, a kindergarten combined with a sports facility and a new guest house, were opened.

The kindergarten was first designed for 15 children aged 2 to 7 years. In 2012, its capacity was enlarged to accommodate 20 children. The kindergarten has a spacious playroom and two well-equipped outdoor playgrounds.
The sports facility (squash court and fitness) serves the entire Academy campus. The gym is provided with all basic training equipment.

An extensive reconstruction of Pavilion V, which should house the new infrastructure supporting basic research in the area of chemical biology and genetics (project CZ-OPENSCREEN), started in 2012.

From the left: main IMG building, kindergarten, guest house (far back: animal facility)

Entrance to the sports facility (left) and kindergarten (right)

New instruments

- Integrated robotic system for high-throughput screening
- Polychromatic high-speed cell sorter
- Fully automated independent slide staining system
- Suspension array system for protein and nucleic acid research
- Small animal imaging system for preclinical research
- Individually ventilated cages; bedding dispensing system; semi-automated cage washer
- Facility for research of marine model organisms maintaining the embryos and adults in temperature-, light-, salinity-, and pH-controlled conditions, using natural sea water obtained regularly from the North Sea
- New IT equipment for data storage, backups and archiving
- Microwave Internet link to the detached site in Vestec planned for the BIOCEV project

New web pages, visual identity of the Institute

The new website of the Institute was launched in September 2012 with significant changes in its structure and appearance.
The new website also represents a final step in the transition to the new visual identity of the Institute after the selection of a new logo of the Institute and the creation of the visual identity manual in 2010.

Projects of EU-Funded Operational Programmes (Structural Funds ERDF, ESF)

Operational Programme Research and Development for Innovations

BIOCEV

The BIOCEV project is one of the large research projects aimed at the establishment of 'European Centres of Excellence' funded by the Ministry of Education, Youth and Sports of the Czech Republic via the Operational Programme Research and Development for Innovations (OP R&DI). The project comprises construction of a complex of research buildings of more than 26,000 m2 floor space, equipped with state-of-the-art instruments and technologies for biotechnological and biomedical research, which should house almost 600 researchers and students.

The name of the project is an acronym of 'Biotechnology and Biomedicine Centre in Vestec'; the village Vestec is located at the south-eastern edge of Prague, approx. 8 km from the Krč campus of the Academy of Sciences. The construction site is close to several biotech companies previously spun-off from IMG.

The project is based on three 'pillars': top research integrated in synergistic research programmes, training of undergraduate and graduate students, and effective transfer of research results into practice. It is a joint enterprise of a consortium of six institutes of the Academy of Sciences of the Czech Republic (the Institute of Molecular Genetics, the Institute of Biotechnology, the Institute of Microbiology, the Institute of Physiology, the Institute of Experimental Medicine, and the Institute of Macromolecular Chemistry) and two faculties of Charles University in Prague (the Faculty of Science and First Faculty of Medicine). It is managed by the BIOCEV Board composed of the representatives of all these institutions. The official guarantor of the project and recipient of the funding of almost CZK 2.3 billion is IMG. Based on completed project documentation, in July 2011 the project obtained the building authorization and in October 2011 the project was approved by the European Commission. On January 31st, 2012, the definite approval by the Czech Ministry of Education, Youth and Sports was granted. The end of 2012 was the term of the tender for the building contractor (the building should be completed by the end of 2014) and the tenders for scientific instruments and technologies. The Director of the project (Pavel Martásek) was selected in an international competition in November 2012. The technical and administrative team responsible for the management of the project is headed by Jan Zemánek. The IMG Deputy Director for the BIOCEV project is Radislav Sedláček; the internal IMG Project Manager is Jitka Černá.

More details on the research programmes can be found at the project website.

www.biocev.eu/en/research-programme/

Both basic and applied biotechnological and biomedical research will be conducted in the BIOCEV project in five synergistic research programmes comprising more than 50 research teams:

- Functional Genomics
- Cellular Biology and Virology
- Structural Biology and Protein Engineering
- Biomaterials and Tissue Engineering
- Development of Diagnostic and Therapeutic Procedures

BIOCEV

The main interest of IMG lies in the first of these programmes (Functional Genomics), which is functionally interconnected with the research infrastructure of the "Czech Centre for Phenogenomics" (CCP or known also as 'Mouse Clinic'), a facility that participates in the worldwide network of similar facilities with the ambition to describe functions of more than 20 thousand of mouse genes in the next ten years. This programme, which was launched as the first BIOCEV research programme in August 2012, is headed by Radislav Sedláček.

ESFRI Research Infrastructures involved in the BIOCEV project

Euro-BioImaging

The Research centre BIOCEV participates as an associated partner in an important European consortium – Euro-BioImaging.

Euro-BioImaging is a large-scale pan-European research infrastructure project on the European Strategy Forum on Research Infrastructures (ESFRI) Roadmap. Euro-BioImaging will deploy a distributed biological and biomedical imaging infrastructure in Europe in a coordinated and harmonized manner. By providing access to and training in imaging technologies, and by sharing of best practice and image data, Euro-BioImaging will become an engine that will drive European innovation in imaging research and technologies.

The preparatory phase of the project started on 10th December 2010. During this phase (2010 – 2013), Euro-BioImaging will develop construction and operation plans of a future large research infrastructure of closely interconnected imaging facilities in Europe.
Pavel Hozák serves as an appointed national representative of the Czech Republic, and he coordinates Work Package 7 – Training in the Euro-Bioimaging project.

www.eurobioimaging.eu

Infrafrontier

Infrafrontier is "The European Infrastructure for Phenotyping and Archiving of Model Mammalian Genomes". The goal of the consortium Infrafrontier is to define the function of all mouse genes and thus model the gene functions in man. As mouse and man share about 95 % of their genetic information, in this respect the mouse represents an excellent model for the study of the function of human diseases.

Radislav Sedláček is a member of the Board of Directors of Infrafrontier and the leader of the Czech node.

www.infrafrontier.eu

Operational Programme Education for Competitiveness

Three projects associated with the implementation of the BIOCEV project have been launched in recent years to support the working programme 1 (Functional Genomics) headed by Radislav Sedláček:

Formation of an expert team of the "Czech Centre of Phenogenomics"– started in April 2011;
Establishment of the Centre of Transgenic Technologies – started in April 2012;
Founding the expert platform for phenotyping and imaging technologies – started in December 2012.

Operational Programme Prague for Competitiveness

Operational Programme Prague - Competitiveness
European Regional Development Fund
Prague & EU: Investing in your future

CZ-OPENSCREEN

CZ-OPENSCREEN – National Infrastructure for Chemical Biology. The purpose of the project, which started in March 2011, is construction of the infrastructure that will support basic research in the area of chemical biology and genetics and offer Open Access to academic researchers. This infrastructure is being equipped with state-of-the-art technology that includes an integrated robotic system for high-throughput screening, a system for automated microscopic high-content analysis and an integrated robotic system for compound storage and management. The mission of the project is to set up a national infrastructure for chemical biology which includes the national compound collection and the database that enable identification of research tools and probes to be used in basic research and in development of potential therapeutics. CZ-OPENSCREEN is a priority project in The National Roadmap of the Large Infrastructures and will serve as a National node within the ESFRI infrastructure EU-OPENSCREEN.

Petr Bartůněk is the National Coordinator of CZ-OPENSCREEN.

www.eu-openscreen.eu

Label-free Technology Platform

The project started in September 2012 and should provide equipment for non-invasive real-time monitoring of changes in the living systems with the aim to identify molecules that could be used as tools for further biomedical research or in drug development process. The purchased instruments will supplement the current laboratory equipment within the National infrastructure for chemical biology CZ-OPENSCREEN and IMG.

Petr Bartůněk is the Coordinator of the project.

Awards & Honours

www.img.cas.cz/achievements/awards-and-honours/

2011

Laboratory of Jiří Bartek (Zdeněk Hodný, Lenka Rossmeislová, Hana Hanzlíková, Kateřina Krejčíková, Markéta Vančurová) – Prize of the Academy of Sciences of the Czech Republic
Jarmila Hnilicová – Josef Hlávka Award for the best students
Petr Heneberg – Prize of the Czech Immunological Society for the best scientific publication by a young immunologist
Libor Macůrek – Otto Wichterle Award for outstanding young scientists
Marie Lipoldová – ARPA-CIS Milan Pospíšil Award 2011
Zuzana Hájková – Prize of the Dean of the Faculty of Science, Charles University Prague, for the best diploma thesis

2012

Václav Pačes – Honorary Doctorate Degree at the Institute of Chemical Technology, Prague
Matyáš Šíma – 1st Michael Boubelík Award of the Society for Laboratory Animal Science

Seminars & Conferences

www.img.cas.cz/events/

Conferences and Courses

2011

03/06	4th IMG PhD Conference
13-17/06	Transmission Electron Microscopy in Life Sciences (a theoretical and practical course for beginner and intermediate students)
31/10-10/11	35th Advances in Molecular Biology and Genetics
11/11	RNA Club 2011
16/12	Annual IMG Conference

2012

08/06	5th IMG PhD Conference
15-19/10	Image Acquisition and Processing in Microscopy (a 5-day theoretical course with demonstrations and practical training)
5-16/11	36th Advances in Molecular Biology and Genetics
27/11	Microscopic Immunodetection in Biomedicine
29/11-1/12	Advanced Techniques in Fluorescence Microscopy (a practical course)
14/12	Annual IMG Conference

Regular weekly Institute seminars – IMG speakers

2011

Pavel Otáhal
Romana Bořkovcová (Kučerová)
Stanislav Vinopal
Jakub Rídl
Michaela Starostová
Karel Chalupský
Petr Pachl
Filip Šenigl
Pavel Moudrý
Ondřej Svoboda
Ondřej Ballek
Eliška Svobodová
Kamila Burdová
Zuzana Cvačková
David Homolka
Jan Dvořák
Vendula Pospíchalová
Tomáš Venit
Jana Šáchová
Lucie Klímová
Ondřej Štěpánek

2012

Markéta Černohorská
Kateřina Podolská
Jan Švadlenka
Aleš Neuwirth
Martin Košař
Antonio Pombinho
Kateřina Trejbalová
Eva Dušková
Petr Flachs
Viktor Bugajev
Vladimír Čermák
Petr Těšina
Václav Urban
Peter Trošan
Milan Reiniš
Miluše Hroudová
Jana Nejepínská

PhD Programme

www.img.cas.cz/phd

Students represent a significant component of our scientific community; about 100 PhD students (20 % international from both EU and non-EU countries) 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 programme is based on the PhD programmes of Prague universities, mainly Charles University and the Institute of Chemical Technology. The PhD programme and related topics are organized by our PhD Committee, which consists of four PIs (Dominik Filipp, Zbyněk Kozmík, David Staněk, Petr Svoboda) and two student representatives (Michaela Liegertová and Tomáš Venit). In addition to everyday contact with their supervisors, students submit two reports to the Committee (in the second and fourth year) about their projects. This provides them with an external feedback and helps them to finish their studies on time. Further education is arranged through a number of lectures and courses organized by scientists from the Institute. PhD students also actively participate in lab meetings, journal clubs, and Institute seminars. Students can also attend English language classes, which take place directly in the IMG building.

Students apply to the programme through an on-line application. In 2011 and 2012, about 20 candidates were selected each year and invited for a PhD interview. The applicants gave a short presentation of their diploma thesis research in English and were briefly interviewed and assessed by a three-member committee. During the interview the applicants also visited selected laboratories and met with lab group leaders in order to find the best match. At the end, twelve students were recruited during the PhD interview procedure in 2011 and eleven in 2012.

We also aim to foster extracurricular training of our PhD students. Since 2010, we have organized a "Welcome Weekend" for the new PhD students in Lštění, the training centre of the Institute located in southern Bohemia, where they are provided with basic information about the Institute and the PhD programme. Since 2008, PhD students have also organized annual IMG PhD conferences. These have established a nice tradition of students and researchers coming together in an informal atmosphere to listen to both student talks and keynote lectures given by invited speakers.

Sports Facility

www.img.cas.cz/other-facilities/sports-facility/

The sports facility (squash court and fitness centre), which started to operate at the beginning of 2011, is situated in the building of the new IMG kindergarten close to the main IMG building. Besides IMG employees, it is available to all people working on campus. The fitness centre is equipped with a cross trainer, two exercise bikes, a bench multi-press, a wall ladder with a horizontal bar for exercising the abdominal muscles, a peck-deck machine, an upper and lower pulley for exercising the muscles of the back, a tricep pulley, an inverse pulley, a positional bench, a set of free weights ranging from 2.5 kg to 25 kg, and an adjustable dumbbell.

Guest House

www.img.cas.cz/other-facilities/guesthouse/

The new guest house of the IMG, also opened in 2011, is a small two-storey building located in a quiet environment, adjacent to the kindergarten. In front of the guest house there is a small parking lot, on the other side there is an outside terrace overlooking the greenery.

Eleven rooms are available for accommodation – mostly studios. The largest unit consists of two rooms, one of which includes a kitchen area. All accommodation units have a small hallway and private bathroom, and the rooms are furnished. The kitchens are equipped with all basic appliances. Internet access is available in all rooms.

All occupants have access to a common laundry room. The maintenance and repairs of the accommodation units are provided, together with cleaning of all common areas.

Kindergarten

www.img.cas.cz/en/other-facilities/kindergarten/

The kindergarten started to operate in January 2011 in a new building adjacent to the main building of the IMG. Its present capacity is 20 children aged between 2 and 7 years. It is operated by a professional company "Kindergarten of the AS CR, Ltd."

In May 2012, the IMG was awarded funding for the project "Kindergarten of the Academy of Sciences of the Czech Republic" within the framework of the Operational Programme Prague – Adaptability (OPPA), Priority Axis 2 – Support of Access to the Labour Market. The project is financed by the European Social Fund (ESF) and the funding is provided by the Municipality of Prague. The project is implemented with the help of the company Kindergarten AS CR, Ltd., which, as a project partner, is in charge of the practical matters during the entire duration of the project. The project should be concluded in October 2014.

The main goal of the project is to enable parents to easily return to work. It considerably alleviates the current problem with insufficient capacity of state-run kindergartens.
The project introduces many new activities that will promote comfort and professionalism of the kindergarten. The purchase of a piano, construction of a new playground and launch of a new website at www.msakademieved.cz are some of the new innovations. September 2012 saw the first regular issue of the quarterly "Kindergarten – Basis for Life". The teachers prepare a portfolio for each child: a journal containing basic information about the child, samples of his/her art and other works, a record of his/her progress, development and improvement, photos, etc. A speech therapist, optometrist and other specialists are scheduled to visit regularly.

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Diploma Theses 2011

Antošová Barbora	Characterization of mice with constitutively active Wnt/beta-catenin signaling pathway in lens (Supervisor: Zbyněk Kozmik; Faculty of Science, Charles University in Prague)
Buryova Halka	Characterization of the role of SPINK6 in the epidermis using transgenic models (Supervisor: Radislav Sedláček; Faculty of Science, Charles University in Prague)
Dobeš Jan	The study on the physiological importance of enteric alpha-defensin expression in the thymus (Supervisor: Dominik Filipp; Faculty of Science, Charles University in Prague)
Eitler Jiří	Involvement of asthma-associated protein ORMDL3 in mast cell signalling (Supervisor: Petr Dráber; Faculty of Science, Charles University in Prague)
Gurská Daniela	The functional analysis of invertebrate promoters in heterologous systems (Supervisor: Zbyněk Kozmik; Faculty of Science, Charles University in Prague)
Hájková Zuzana	The effect of mast cell activation on microtubule organization (Supervisor: Pavel Dráber; Faculty of Science, Charles University in Prague) - Dean Award for the Best Diploma Thesis
Hauserová Viola	Spliceosome assembly (Supervisor: David Staněk; Faculty of Science, Charles University in Prague)
Novák Jakub	Analysis of regulation of cytoplasmic poly(A) polymerase complexes (Supervisor: David Staněk; Faculty of Science, Charles University in Prague)
Oltová Jana	Mapping of regulatory elements within 5' region of the Disp3 locus (Supervisor: Petr Bartůněk; Faculty of Science, Charles University in Prague)
Písačková Jana	Monoclonal antibodies and characterization of their antigen-binding properties (Supervisor: Pavlína Řezáčová; Faculty of Science, Charles University in Prague)
Šimandlová Jitka	Characterization of antirecombinase activity of human FBH1 helicase (Supervisor: Pavel Janščák; Faculty of Science, Charles University in Prague)
Vágnerová Lenka	The use of CAM assay for characterization and study of cancer cell invasive properties (Supervisor: Michal Dvořák; Faculty of Science, Charles University in Prague)

Diploma Theses 2012

Hanusová Zdenka	Physiological role of SIGIRR in early embryonic development (Supervisor: Dominik Filipp; Faculty of Science, Charles University in Prague)
Hradilová Naďa	Role of endocytosis and endosomal acidification in TRAIL-induced apoptosis (Supervisor: Ladislav Anděra; Faculty of Science, Charles University in Prague)
Icha Jaroslav	The role of acetylation in the RNA recognition motif of SRSF5 (Supervisor: David Staněk; Faculty of Science, Charles University in Prague)
Knopf Corinna	Genetic analysis of genomic recombination rate (Supervisor: Jiří Forejt; Veterinärmedizinische Universität Wien)
Moravec Martin	Analysis of pluripotent gene expression program in early embryos and embryonic stem cells (Supervisor: Petr Svoboda; Faculty of Science, Charles University in Prague)
Pavlíková Michaela	The role of selected cell populations and molecules in inflammatory reaction and rejection of skin allograft (Supervisor: Magdaléna Krulová; Faculty of Science, Charles University in Prague)
Peřinová Lucie	Modulation of properties of mesenchymal stem cells and their use for regulation of transplantation immunity (Supervisor: Magdaléna Krulová; Faculty of Science, Charles University in Prague)
Žlabová Anna	Characterisation of the cell line TRAMP-C2, murine model of prostate cancer (Supervisor: Milan Reiniš; Faculty of Science, Charles University in Prague)

PhD Theses 2011

Brauer Rena	Key role for matrix metalloproteinases-19 and -28 in maintenance of intestinal homeostasis [Supervisor: Radislav Sedláček; Faculty of Medicine, University of Kiel]
Dráber Peter	Mechanisms of signal transduction by leukocyte surface receptors and transmembrane adaptor proteins [Supervisor: Tomáš Brdička; Faculty of Science, Charles University in Prague]
Dupačová Naoko	The role of Wnt signaling in embryonic development [Supervisor: Zbyněk Kozmik; Faculty of Science, Charles University in Prague]
Dzijak Rastislav	Nuclear dynamics and interactions of myosin 1c [Supervisor: Pavel Hozák; Faculty of Science, Charles University in Prague]
Grekov Igor	Experimental murine leishmaniasis and its application for drug discovery and study of pathogen-host interactions [Supervisor: M. Lipoldová; Third Faculty of Medicine, Charles University in Prague]
Hnilicová Jarmila	Regulation of splicing in the context of the cell nucleus [Supervisor: David Staněk; Faculty of Science, Charles University in Prague]
Hořejší Barbora	Tubulin isotypes in cancerogenesis [Supervisor: Pavel Dráber; Faculty of Science, Charles University in Prague]
Klíma Martin	Molecular and functional characterization of the death receptor 6 [Supervisor: Ladislav Anděra; Faculty of Science, Charles University in Prague]
Kobets Tetyana	Methods for Leishmania parasites detection and quantification as a tool for study of pathogen-vector-host interactions [Supervisor: Marie Lipoldová; Third Faculty of Medicine, Charles University in Prague]
Matoušková Magda	Mechanisms of endogenous retrovirus control in the host cell [Supervisor: Jiří Hejnar; Faculty of Science, Charles University in Prague]
Novotný Ivan	Formation of spliceosomal snRNPs in the cell nucleus [Supervisor: David Staněk; Faculty of Science, Charles University in Prague]
Ormsby Tereza	Studies on immunoreceptor signaling molecules [Supervisor: Václav Hořejší; Faculty of Science, Charles University in Prague]
Procházková Jana	The role of cytokines in development and differentiation of regulatory T cells [Supervisor: Vladimír Holáň; Faculty Science, Charles University in Prague]
Rohožková Jana	Molecular determination of plant/Potyvirus interaction. [Supervisor: Milan Navrátil; Faculty of Science, Palacký University, Olomouc]
Štěpánek Ondřej	Regulation of signal transduction by leukocyte surface proteins [Supervisor: Tomáš Brdička; Faculty of Science, Charles University in Prague]

PhD Theses 2012

Fafílek Bohumil	Adjusting Wnt signalling – new regulatory mechanisms of the Wnt pathway [Supervisor: Vladimír Kořínek; Faculty of Science, Charles University in Prague]
Flemr Matyáš	mRNA stability and microRNA activity in mouse oocytes. [Supervisor: Petr Svoboda; Faculty of Science, Charles University in Prague]
Homolka David	Meiotic sex chromosome inactivation in mouse spermatogenesis [Supervisor Petr Jansa; Faculty of Science, Charles University in Prague]
Hrdinka Matouš	The role of membrane microdomains and transmembrane adaptor proteins PRR7 and SCIMP in the regulation of immunoreceptor signaling [Supervisor: Karel Drbal; Faculty of Science, Charles University in Prague]
Hroudová Miluše	Study of homeobox genes of the sweet water medusa Craspedacusta sowerbyi [Supervisor: Václav Pačes; Institute of Chemical Technology Prague]
Hubáčková Soňa	DNA damage and signalling pathways in cellular senescence [Supervisor: Zdeněk Hodný; Faculty of Science, Charles University in Prague]
Kumpošt Jiří	Metabotropic glutamate receptors and their associated proteins involved in signaling [Supervisor: Jaroslav Blahoš; Faculty of Science, Charles University in Prague]
Moserová Irena	Molecular mechanisms of apoptosis induced by photodynamic activation in cancer cells [Supervisor: Jarmila Králová, Faculty of Science, Charles University in Prague]
Moudrý Pavel	Posttranslational modifications in DNA damage response [Supervisor: Zdeněk Hodný; Faculty of Science, Charles University in Prague]
Neuwirth Aleš	Defensins and autoimmunity: emerging α-defensin based models to study mechanisms underpinning autoimmune processes [Supervisor: Dominik Filipp; Faculty of Science, Charles University in Prague]
Pajer Petr	From the search for new oncogenes to the effort of redefining the cancerogenesis phenomenon [Supervisor: Michal Dvořák, Faculty of Science, Charles University in Prague]
Pokorná Kateřina	Pre-clinical model of acute promyelocytic leukemia: Study of the anti-leukemic effect induced by ATRA and DNA vaccination [Supervisor: Vladimír Holáň; Faculty of Science, Charles University in Prague]
Pospíchalová Vendula	Regulatory mechanisms of Wnt signalling [Supervisor: Vladimír Kořínek; Faculty of Science, Charles University in Prague]
Tůmová Magda	Regulatory functions of the transmembrane adaptor protein NTAL in activation of mast cells [Supervisor: Petr Dráber; Faculty of Science, Charles University in Prague]
Vinopal Stanislav	Functional characterization of selected microtubule regulatory proteins [Supervisor: Pavel Dráber; Faculty of Science, Charles University in Prague]

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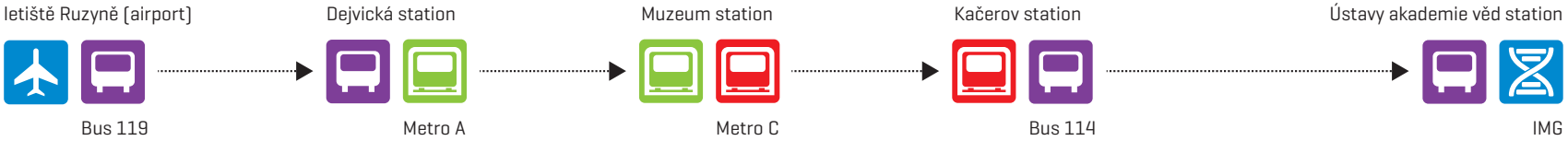

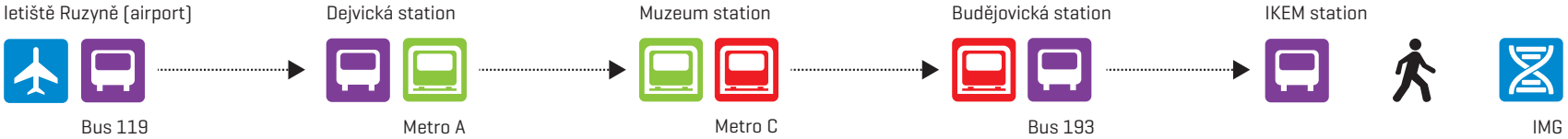

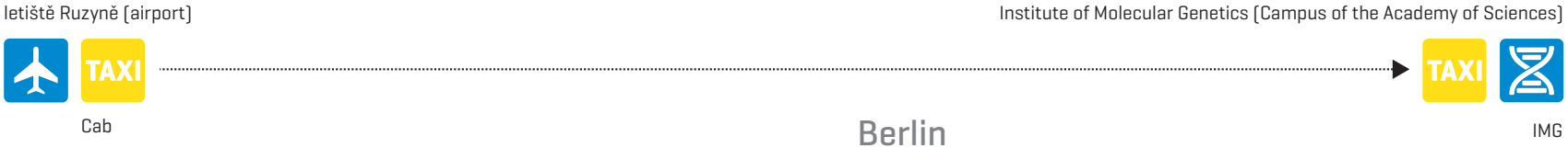

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