IMG

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

Annual Report

2013-2014

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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 conference hall (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 several events organized also by other on-campus institutes of the Academy.

We opened a newly reconstructed Pavilion V (the reconstruction lasted for two years) which hosts a new infrastructure supporting basic research in the area of chemical biology and genetics (CZ-OPENSCREEN: National Infrastructure for Chemical Biology, project OPENSCREEN).

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, 23 research groups of the Institute are dealing with the topics covering molecular and cellular immunology, molecular and cellular oncology, cell biology of the nucleus, cytoskeleton, functional genomics and bioinformatics, study of oncogenes, molecular biology of development, structural biology and mechanisms of receptor signalling. Students represent a significant component of our scientific community; 80 doctoral students and 50 undergraduate students work on 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.

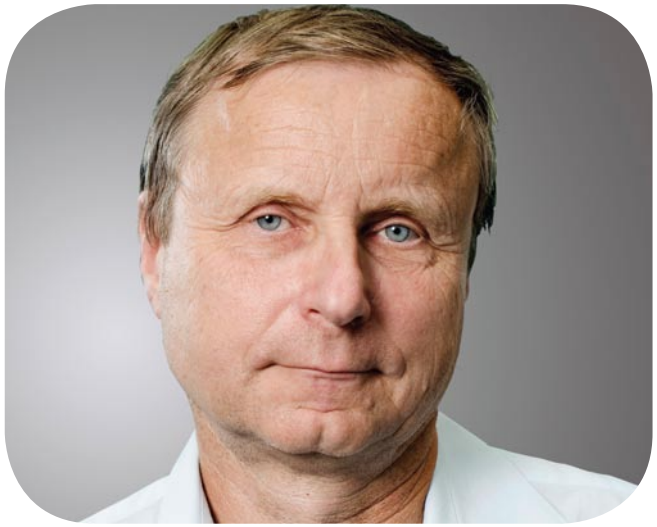
In 2013-14, the Institute scientists were again authors or co-authors of publications in a number of prestigious international journals including Nature, Cell, Proceedings of the National Academy of Sciences of the USA, Journal of Cell Biology, Blood, Current Biology, Nucleic Acids Research, Development,

Gastroenterology, Cancer Research, Oncogene, Cell Death and Differentiation, Journal of Immunology, European Journal of Immunology, Molecular and Cellular Biology, Molecular Biology of the Cell, Journal of Virology, Journal of Cell Science, International Journal of Cancer, Cell Cycle, Journal of Medicinal Chemistry, Molecular Biology of the Cell, Journal of Biological Chemistry, PLoS Genetics, Cellular Signalling, etc.

The excellence of the Institute researchers is confirmed by awards and prizes. In 2013 Jiří Bártek received the František Běhounek Award for excellent representation of the Czech Republic in European Research and Development, Jiří Bártek and Václav Hořejší were awarded the Silver Memorial Medal of the Senate of the Parliament of the Czech Republic for their scientific achievements, in 2014 Petr Svoboda was awarded The Neuron Fund Prize in the field of medicine, Jiří Hejnar and his team received Prize of the Academy of Sciences of the Czech Republic, Matyáš Flemr received Discovery Award from company Novartis for basic research in biomedicine.

Of crucial importance for the Institute is the BIOCEV project supported from the Operational Programme Research & Development for Innovations (www.biocev.eu). The building in the nearby village Vestec will be finished, equipped and fully operational in second half of 2015.

Despite the funding stagnation in recent years, 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

Brief History

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

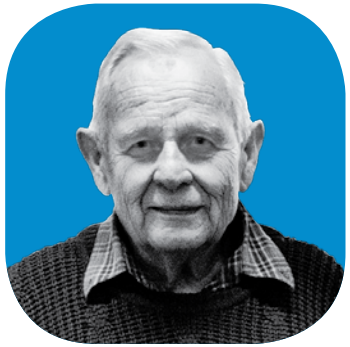
The Institute of Molecular Genetics, Academy of Sciences of the Czech Republic (IMG), is located on the southern outskirts of Prague, capital of the Czech Republic.

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 400 employees and students.

Milan Hašek
Director 1962 – 1970

Prokop Málek
Director 1970 – 1977

Josef Říman
Director 1977 - 1991



Jan Svoboda
Director 1991 - 1999

Václav Pačes
Director 1999 – 2005

Václav Hořejší
Director 2005 – 2017

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 research 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.

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
Chairman of the Supervisory Board

Vladimír Kořínek
Chairman of the Institute Council

Ilona Dita
Institute Secretary

Laboratory of Cell Signalling and Apoptosis

Apoptosis, death receptors, TRAIL, senescence, cancer

Ladislav Anděra

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The research interest of our group is focused on the analysis and characterization of death signalling mainly in cancer cells, preferably induced by the activated death receptors (DRs) of the TNFR family such as TRAIL receptors TRAIL-R1/DR4, TRAIL-R2/DR5 and Fas/CD95. DR-triggered apoptotic signalling relies on ligand-dependent assembly of the multiprotein Death-Inducing Signalling Complex (DISC) and subsequent proximity-induced self-processing and activation of the initiator caspase-8. Activated caspase-8 then cleaves a group of its target proteins, notably the effector procaspase-3 and the BH3-only protein Bid. The processed caspase-3 subsequently cleaves a number of cellular structural and functional proteins and plays an important role in ordered disassembly of the apoptotic cell. Truncated Bid processed by caspase-8 (tBid) moves from the cytoplasm to mitochondria, where it participates in the activation of Bax/Bak-mediated permeabilization of the mitochondrial outer membrane and release of pro-apoptotic proteins such as cytochrome c, Smac or AIF. Our recent findings in the death receptor signalling include uncovering the endosomal acidification occurring during endocytosis of activated TRAIL receptors as an enhancer of caspase-8 processing and activation (Horova et al., 2013), and the discovery of plant alkaloid homoharringtonine (HHT) as an enhancer of TRAIL-induced apoptosis of resistant colorectal carcinoma cells (Beranova et al., 2013). The HHT sensitizing impact is largely correlated with HHT-mediated downregulation of expression of antiapoptotic proteins Mcl-1 and cFLIP. In mutual collaboration with the Department of Histology and Embryology, MU Brno we analysed and characterized the expression and function of death receptors in human embryonic and induced pluripotent stem cells (Vinarsky et al., 2013). In further collaborations we also contributed to the functional analysis of other enhancers of TRAIL-induced apoptosis of cancer cells such as roscovitine (Molinsky et al., 2013), curcumin, and emetine and to uncovering the participation of activated caspase-8 in non-apoptotic signalling (Somasekharan et al., 2013). Currently, we analyse TRAIL-induced signalling in pancreatic cancer cells, examine the role of individual pro-apoptotic TRAIL receptors TRAIL-R1/DR4 and TRAIL-R2/DR5 in their pro- and non-apoptotic signalling, and evaluate the effect of drug- and cell cycle inhibitor-triggered senescence in cancer cells on their sensitivity to various apoptogens and on the expression and function of crucial proteins participating in the activation and regulation of apoptosis or necroptosis.

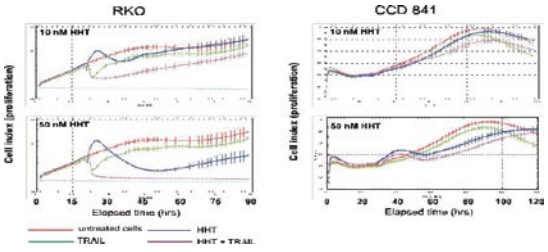

Figure 1. HHT strongly enhances TRAIL-mediated growth suppression of resistant cancer cells.
Colorectal cancer RKO or normal colon epithelial CCD 841 cells were seeded in triplicate into a 96-well E-plate and treated with 100 ng/ml TRAIL, 10 or 50 nM HHT, or their combination in the xCELLigence RTCA analyser (Roche). The cell index representing cell proliferation was measured every 15 minutes. The data represent one out of four experiments with similar outcomes (adapted from Beranova et al., 2013).

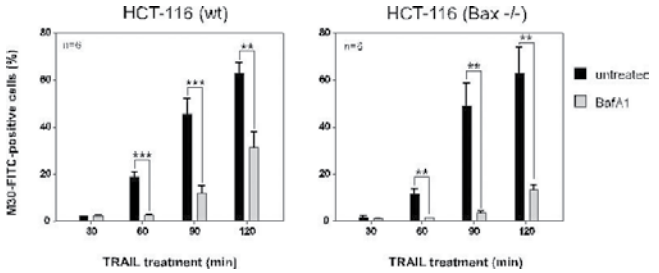

Figure 2. Inhibition of V-ATPase significantly suppresses TRAIL-triggered activation of caspases.
A. HCT-116wt and HCT-116Bax-/- cells, either untreated or pre-treated for 1 hour with V-ATPase inhibitor bafilomycin A1 (BafA1, 20 nM), were incubated with recombinant TRAIL (200 ng/ml) for the indicated time periods, and the fraction of cells with activated caspases was quantified by M30-FITC staining and flow cytometry (adapted from Horova et al., 2013).

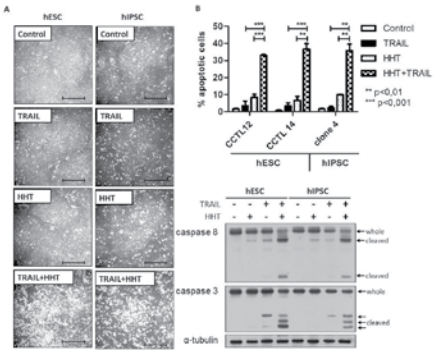

Figure 3. The protein-synthesis inhibitor homoharringtonine (HHT) sensitizes hESC and hiPSC to TRAIL-induced apoptosis.
HESC and hiPSC were either left untreated (control), treated with 50 nM HHT or 200 ng/ml human recombinant TRAIL, or pre-treated with 50nM HHT for 1 hour followed by 200 ng/ml human recombinant TRAIL treatment (HHT+TRAIL). Their morphology (A) was analysed by phase contrast microscopy and the activation and processing of caspases by flow cytometry using an antibody against caspase-3-cleaved PARP (B) or by Western blotting using anti-caspase-8 and -caspase-3 antibodies (C); adapted from Vinarsky et al., 2013.

- MEYS, LH12202 LH-KONTAKT – Analysis and suppression of mechanisms leading to the resistance of cancer cells to TRAIL, 2012-2014, L. Anděra
- MH, NT13201 – Role of angiogenesis in mantle cell lymphoma (MCL) survival, growth, spread and response to therapy, 2012-2015, L. Anděra

1. Beranova L, Pombinho AR, Spegarova J, Koc M, Klanova M, Molinsky J, Klener P, Bartunek P, Andera L: The plant alkaloid and anti-leukemia drug homoharringtonine sensitizes resistant human colorectal carcinoma cells to TRAIL-induced apoptosis via multiple mechanisms. **Apoptosis 2013** 18(6): 739-50.
2. Horova V, Hradilova N, Jelinkova I, Koc M, Svadlenka J, Brazina J, Klima M, Slavik J, Hyrslova Vaculova A, Andera L: Inhibition of vacuolar ATPase attenuates the TRAIL-induced activation of caspase-8 and modulates the trafficking of TRAIL receptosomes. **FEBS J 2013** 280(14): 3436-50.
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4. Somasekharan SP, Koc M, Morizot A, Micheau O, Sorensen PH, Gaide O, Andera L, Martinou JC: TRAIL promotes membrane blebbing, detachment and migration of cells displaying a dysfunctional intrinsic pathway of apoptosis. **Apoptosis 2013** 18(3): 324-36.
5. Vinarsky V, Krivanek J, Rankel L, Nahacka Z, Barta T, Jaros J, Andera L, Hampl A: Human embryonic and induced pluripotent stem cells express TRAIL receptors and can be sensitized to TRAIL-induced apoptosis. **Stem Cells Dev 2013** 22(22): 2964-74.

From the left: Simona Benešová / Technician, Jan Švadlenka, MSc / PhD Student, Jan Bražina, MSc / PhD Student, Zuzana Nahácka, MSc / PhD Student, Ladislav Anděra, PhD / Head of Laboratory, Gita Nováková (until 2014) / Diploma Student

Not in the picture: Marie Ksandrová / Undergraduate Student, Lenka Beranová, MSc / PhD Student (maternity leave), Vladimíra Horová, MSc (until 2013) / PhD Student, Jarmila Špegárová, PhD (until 2014) / Postdoctoral Fellow, Martin Peterka (until 2013) / Diploma Student, František Pešina (until 2014) / Diploma Student

Laboratory of Genome Integrity

DNA damage response, inflammatory cytokines, cellular senescence, RecQ helicases, R-loops

Jiří Bartek

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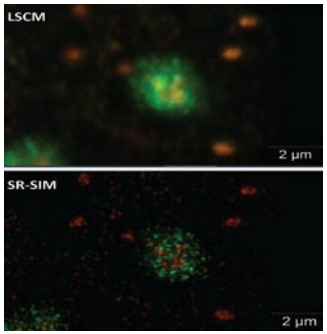
Realization of complex tasks of living organisms depends on the information stored in DNA of their genomes. The loss of this information due to endogenous and exogenous physicochemical damage to DNA results in disintegration of homeostasis at the cellular and organism levels manifested as diseases, including 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 PARylation) of key players involved in sensing and transmitting signals from DNA breaks to cellular effectors involved in activation of cell cycle checkpoints, DNA repair and cell reprogramming; 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, cancer microenvironment and cell reprogramming; 5) exact DNA transactions mediated by RecQ DNA helicases, key players in the maintenance of genomic stability; and 6) 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 identified specific cytokines (IL1β and TGFβ) responsible for genotoxic effects observed in so-called bystander senescence and a mechanism of their action (oxidative stress mediated by elevated expression of NADPH oxidases and downregulation of mitochondrial ATP/ADP translocase type II). We have identified signalling pathways (MAPK and Akt) responsible for radio- and chemo-resistance of prostate cancer cells. Recently, we started a new project aiming to study the molecular mechanisms underlying formation and resolution of RNA:DNA hybrids (R-loops), highly genotoxic structures that can arise as a consequence of collisions between replication and transcription machineries. We focused on identification of proteins associated with R-loops under the conditions of chemically- and oncogene-induced replication stress and studying their role in maintenance of genome stability.

Fig. 1. Super-resolution microscopy of DNA damage lesion. Comparison of DNA lesion, marked by repair protein 53BP1 (green) with juxtaposed/co-associated PML nuclear body (red), persisting in human fibroblasts 6 days after ionizing irradiation (10 Gy) acquired by structured illumination microscopy (SR-SIM, bottom) and conventional/laser scanning confocal microscopy (LCSM) image (up).



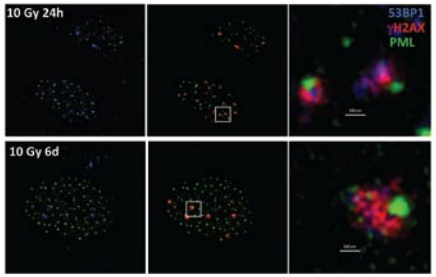


Fig. 2. Super-resolution images of DNA lesions persisting in human fibroblasts 1 (up) and 6 (bottom) days after 10 Gy and marked by established DDR markers gH2AX (red) and 53BP1 (blue) co-associated with PML nuclear body (green).

- GACR, GAP305/10/0281 – Role of the Rothmund-Thomson syndrome gene product in maintenance of genomic stability, 2010-2014, P. Janščák
- GACR, GA13-17658S – Mechanisms of radioresistance of prostate cancer cells, 2013-2016, Z. Hodný
- GACR, GA13-17555S – Premature cellular senescence: Mechanisms and links with cancer, 2013-2016, J. Bártek
- MH, NT14174 – The role of 5-azacytidine in immunoepigenetics and genotoxic stress in the treatment of myelodysplastic syndrome., 2013-2015, J. Bártek
- GACR, 14-05743S – Molecular mechanism of genomic instability caused by oncogene activation, 2014-2016, P. Janščák
- MEYS, LH14037 – Identification of protein complexes associated with genotoxic RNA: DNA hybrids and their role in maintenance of genomic stability, 2014-2016, J. Dobrovolná
- GACR, GA204/09/0565 – Role of RECQ5 DNA helicase in maintenance of genomic stability, 2009-2013, P. Janščák
- GACR, GPP305/11/P683 – Post-translational modifications of Daxx and their functional relevance in DNA damage response and cellular senescence, 2011-2013, H. Hanzlíková

1. Kyjacova L, Hubackova S, Krejcikova K, Strauss R, Hanzlikova H, Dzijak R, Imrichova T, Simova J, Reinis M, Bartek J, Hodny Z: Radiotherapy-induced plasticity of prostate cancer mobilizes stem-like non-adherent, Erk signaling-dependent cells. **Cell Death Differ 2014** doi: 10.1038/cdd.2014.97.
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5. Evangelou K, Bartkova J, Kotsinas A, Pateras IS, Liontos M, Velimezi G, Kosar M, Liloglou T, Trougakos IP, Dyrskjot L, Andersen CL, Papaioannou M, Drosos Y, Papafotiou G, Hodny Z, Sosa-Pineda B, Wu XR, Klinakis A, Ørntoft T, Lukas J, Bartek J, Gorgoulis VG: The DNA damage checkpoint precedes activation of ARF in response to escalating oncogenic stress during tumorigenesis. **Cell Death Differ 2013** 20(11): 1485-97.

From the top left: Rastislav Dzijak, PhD / Postdoctoral Fellow, Jana Dobrovolná, PhD / Research Associate, Soňa Hubáčková, PhD / Postdoctoral Fellow (since 2013), Blanka Gabajová, MSc / PhD Student (since 2013), Hana Hanzlíková, PhD / Research Associate (until 2014), Václav Urban, MSc / PhD Student Terezie Imrichová, MSc / PhD Student (since 2013), Kateřina Krejčíková, MSc / Research Assistant, Michal Šrámek / Technician (since 2013), Monika Žárská, MSc / Research Assistant, Eliška Davidová / Diploma Student, Markéta Vančurová , MSc / Research Assistant, Alena Kučerová / Diploma Student (since 2014) Lucie Knoblochová / Diploma Student (since 2013), Lenka Kyjacová, MSc / PhD Student, Edita Křížová, MSc / PhD Student (since 2014), Zdeněk Hodný, MD, PhD / Deputy Head

Not in the picture: Prof Jiří Bártek MD, PhD / Head of Laboratory, Pavel Janščák , PhD / Research Fellow, Kamila Burdová, MSc / PhD Student (until 2014), Alena Moudrá, MSc / PhD Student (since 2013), Irina Cheveleva, MSc / Research Assistant, Gita Nováková, MSc / PhD Student (since 2014), Filip Havel, MSc / Research Assistant (since 2013), Jana Fryzelková / Diploma Student, Simona Moravcová, PhD / Postdoctoral Fellow (since 2013), Filip Novotný, MSc / Research Assistant, Jan Proška, MSc / Research Assistant (until 2014), Lucie Štolcová, MSc / Research Assistant (until 2014), Lenka Pišlová / Secretary, Martin Košař, MSc / PhD Student (until 2014), Polina Zjablovskaja, MSc / PhD Student (until 2013), Terezie Imrichová MSc / PhD Student (since 2013, Radka Bokorová, MSc / PhD Student (until 2013), Veronika Machalová / Diploma Student (until 2014), Ján Valášek / Diploma Student (until 2014)

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 is study of the molecular mechanism of cell fate determination. We have established in vitro systems to get insight into 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 a more systematic search for such tools using chemical biology approaches.

Recently, we have identified Disp3, a sterol-sensing domain-containing protein. DISP3 (PTCHD2) is predominantly expressed in neural tissues. Ectopic expression of DISP3 in fibroblasts resulted in elevated cholesterol levels combined with an altered cholesterol and lipid distribution (Zikova et al. 2009). We have performed RNAi and overexpression studies of neural stem cell lines and found out that Disp3 is able to modulate the cell fate of neural stem and progenitor cells. We found that ectopically expressed DISP3 promotes cell proliferation and alters expression of genes that are involved in tumorigenesis. Finally, the differentiation profile of DISP3-expressing cells was altered, as evidenced by delayed expression of neural specific markers and a reduced capacity to undergo neural differentiation (Zikova et al. 2014).

We have extended our studies on vertebrate haematopoietic development to the zebrafish model and we have established ex vivo cultures of haematopoietic cells (Stachura et al. 2009). Recently, we have produced several recombinant zebrafish growth factors (Epo, Gcsfa/b, Tpo) that allow us to establish, for the first time, zebrafish haematopoietic clonal assays in semisolid media (Stachura et al. 2011). Granulocyte colony-stimulating factor (Gcsf) drives the proliferation and differentiation of granulocytes, monocytes, and macrophages. Analysis of the zebrafish genome indicates the presence of two Gcsfs, likely resulting from a duplication event in teleost evolution. We show that in addition to supporting myeloid differentiation, zebrafish Gcsf is required for the specification and proliferation of haematopoietic stem and progenitor cells. These findings may bring information on how haematopoietic cytokines had evolved following the diversification of teleosts and mammals from a common ancestor (Stachura et al. 2013). Moreover, these tools enabled us to reveal the clonogenic and proliferation capacity of bi-potent thrombo/erythropoietic progenitors with respect to their mammalian haematopoietic counterparts. Despite obvious phenotypic differences between fish and mammalian thrombocytes and erythrocytes, our results strongly demonstrate the evolutionary conservation of the basic processes and molecular mechanisms of erythro/thrombopoiesis in the vertebrates (Svoboda et al. 2014).

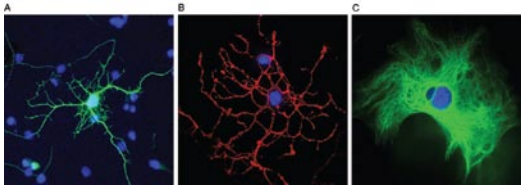

Fig. 1. Differentiation of neural stem cells in vitro
Differentiation of mouse neural stem cells (NS-5 cell line) into (A) neurons (betaIII-tubulin – green) at day 12, (B) oligodendrocytes (O4 - red) at day 9 and (C) astrocytes (GFAP – green) at day 4. Nuclei are stained with DAPI (blue).

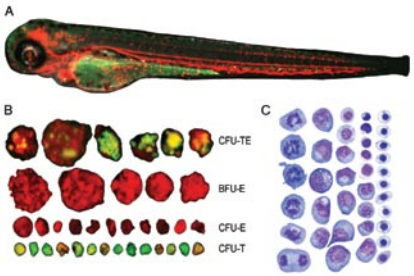

Fig. 2. Zebrafish as a model to study vertebrate haematopoiesis
(A) Double hemizygous transgenic zebrafish Tg(gata1:DsRed); Tg(cd41:EGFP) at 4 days post fertilization with single hematopoietic cells fluorescently labelled (red, erythroid cells, green, thrombocytes). (B) Colonies of hematopoietic cells derived from adult zebrafish whole kidney marrow 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. (C) Morphology of cells isolated from methylcellulose cultures (as in B) after six days in culture were stained with May-Grünwald Giemsa.

- GACR, GAP301/12/1478 – The role of DISP3 protein in lipid metabolism, 2012-2015, P. Bartůněk
- MEYS, LM2011022 – CZ-OPENSCREEN: National Infrastructure for Chemical Biology, 2012-2015, P. Bartůněk
- MIT, FR-TI4/802 – Development of new chemical compounds with anti-tumour activities or use in regenerative medicine, 2012-2015, P. Bartůněk, V. Kořínek
- TACR, TA02010212 – ReceptorX: Integrated platform for drug discovery and development, 2012-2015, P. Bartůněk
- MEYS, LO1220 - CZ-OPENSCREEN – National infrastructure for chemical biology, 2013-2018, P. Bartůněk
- OPPC, CZ.2.16/3.1.00/21547 – Centre for Model Organisms, 2014-2014, P. Bartůněk
- FP7 EU, 261861 EU-OPENSCREEN – European Infrastructure of Open Screening Platforms for Chemical Biology, 2010-2015, P. Bartůněk
- GACR, GAP305/10/0953 – New regulators of megakaryocyte and erythroid lineage commitment, 2010-2013, P. Bartůněk
- OPPC, CZ.2.16/3.1.00/24020 CZ-OPENSCREEN – National Infrastructure for Chemical Biology, 2011-2013, P. Batůněk
- OPPC, CZ.2.16/3.1.00/28026 – Label-free technology platform, 2012-2013, P. Batůněk

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Not in the picture: Tomáš Bartoň, MSc / PhD Student, Jana Bartůňková, MD / Research Assistant, Milan Gottwald, Bc / Research Asssistant (from 2014), Assoc Prof Jindřich Jindřich / Research Fellow, Petr Pajer, PhD / Research Fellow, Assoc Prof Daniel Svozil, PhD / Research Fellow

Laboratory of Molecular Pharmacology

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

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Our research is focused on the structure-function relationship of metabotropic glutamate receptors (mGluRs) and Cannabinoid Receptor 1 (CB1R) signalling. The mGluRs belong to Class C-G-protein Coupled Receptors (GPCRs) and were traditionally viewed as homodimers, composed of two identical subunits. Using the mutagenesis approach combined with a functional expression system we showed that within their dimeric complexes only one subunit reaches the active state. The activation process of mGluRs is initiated by agonist binding that causes conformational changes of the extracellular ligand-binding domains. 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. Recently resolved crystal structure of the mGluR1 is in accord with our model of activation mechanism being asymmetrical, as suggested by our functional data.
Moreover, we showed that splice variants mGluR1a and mGluR1b form heterodimers in vivo. The functional relevance of the splice variant combinations in the dimeric mGluR1 complexes in vivo are now under investigation using genetically modified mice.
Cannabinoid receptors are located predominantly pre-synaptically, where the receptors show modest internalization upon agonist stimulation, while the CB1R expressed in heterologous systems are readily internalized. We detected novel interacting partner of CB1R that modulates internalization of CB1R and also signalling of the receptor in biased manner. Overexpression of SGIP1 in animals is associated with obesity. Functional significance of the SGIP1 protein on CB1R signalling is studied both on level of signalling-pathway specificity, as well as in animal models for energy homeostasis regulation.

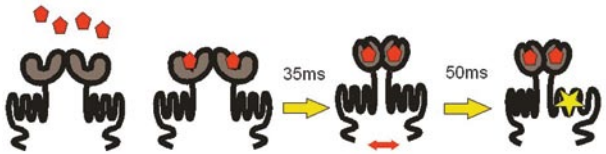

Fig. 1. Activation of Class C GPCR schematically Together with our collaborators we brought evidence that activation of metabotropic glutamate receptors following agonist binding (red pentagons) within extracellular binding sites (also known as Venus fly-trap like domains) results in the change in relative 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. [**EMBO J 2005** 24(3): 499-509 and **Science Signal 2012** 5(237): ra59]

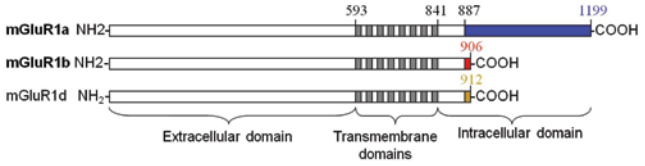

Fig. 2. Long and short variants of metabotropic glutamate receptor 1 Splicing of metabotropic glutamate receptor 1 (mGluR1) gene results in expression of long and short forms. Following the heptahelical domain and short sequence including RRKK motive (Endoplasmic Reticulum retention signal), the long form mGluR1a has unique sequence of 312 aa, short forms are termed mGluR1b and d. The mGluR1b unique sequence is 19 aa long following the splicing site.

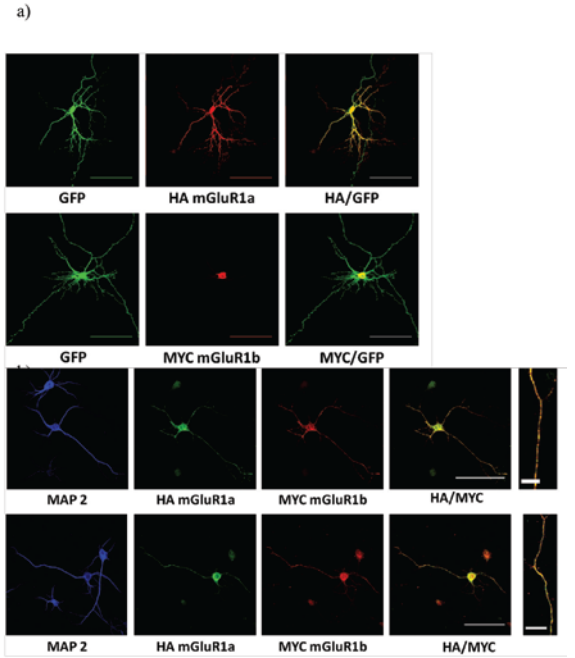

Fig. 3 Splice variants mGluR1a or mGluR1b combine in mGluR1a/b dimers Cells from primary prefrontal cortical neurons were transfected a) with GFP and corresponding single tagged subunits and stained 72 hours later against HA and MYC epitopes or visualized using GFP fluorescence. UPER ROW: Left panel: GFP; middle panel: HA-mGluR1a detected using anti-HA antibodies; right panel-merge. LOWER ROW: left panel: GFP; middle panel MYC-mGluR1b detected using anti-MYC antibodies, right panel: merge; b) Two primary cortical neurons co-transfected with HA-mGluR1a (Green) and MYC-mGluR1b (Red); right panel: merge; dendritic marker MAP2 shown in left panel in blue. Co-localization HA-mGluR1a and MYC-mGluR1b in MAP2 positive distal dendrites is shown in extreme right in detail as merge for green and red channels (HA-mGluR1a and MYC-mGluR1b, respectively). Scale bar is 20 μm.

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From the left: Šárka Techlovská, MSc / PhD Student, Michaela Dvořáková / Diploma Student, Assoc Prof Jaroslav Blahoš, MD, PhD / Head of Laboratory, Alena Hájková, MSc / PhD Student, Daniela Franková / Technician,

Not in the picture: Pavla Hubálková (until 2014), Jayne Nicole Rafferty, PhD / Postdoc

Laboratory of Leukocyte Signalling

Signalling by leukocyte surface receptors, Csk-binding proteins, relationship between signalling and leukocyte-driven pathologies

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The Laboratory of Leukocyte Signalling is studying the molecular mechanisms of signal transduction in leukocytes. Our interest has recently been focused on the interplay between adaptor proteins, Src-family kinases, and related kinase Csk. In addition, we are also involved in the research aiming at uncovering the relationship between signal transduction and leukocyte-driven pathologies. Src-family kinases are tightly controlled enzymes critically involved in the signalling via a number of leukocyte surface receptors such as T-cell and B-cell receptors for antigens. The majority of Src-family kinases are directly associated with cellular membranes through specific sequence motifs at their N-termini. Csk is a major negative regulator of these kinases. However, it lacks any membrane targeting sequences, and therefore it relies on the interactions with membrane-associated adaptor proteins to gain access to Src-family kinases and to efficiently regulate their activity. In the past, we discovered several novel members of this group of adaptor proteins and now we are working on the characterization of their functional a biochemical features. These studies include analysis of transmembrane adaptor termed SCIMP, which interacts with both positive (SLP65/76) and negative (Csk) regulators of signalling. We found that in B cells it is involved in MHCII-dependent reverse signalling and currently we are characterizing its role in the signalling pathways of the receptor for pathogenic fungi Dectin-1 in dendritic cells. Another protein that was discovered during our search for novel Csk-binding proteins is known as PSTPIP2. Importantly, defects in PSTPIP2 expression in mice result in an autoinflammatory disorder characterized by sterile inflammation of bones and skin, which closely resembles the human disease known as chronic recurrent multifocal osteomyelitis. We are now exploring the mechanisms of how Csk and lipid phosphatase SHIP1 (an additional inhibitory enzyme recently identified in our laboratory as a binding partner of PSTPIP2) contribute to the control of inflammation by PSTPIP2. Additional clinically relevant projects include studies of signalling proteins aberrantly expressed in childhood leukaemias (OPAL1) and research on changes in leukocyte signal transduction in patients with common variable immunodeficiency (CVID), both running in collaboration with clinical laboratories.

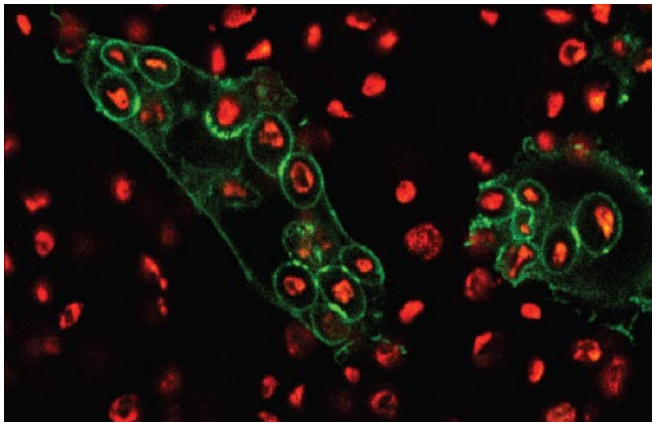

Fig. 3 SCIMP localization in dendritic cells during phagocytosis of Zymosan particles. Phagocytic uptake of Zymosan (consisting of yeast cell wall components) is mediated by Dectin-1 receptor. This image shows localization of SCIMP-EGFP (green) in dendritic cells engulfing Zymosan particles (red). Note the abundant presence of SCIMP in the membranes of Zymosan-containing phagosomes.

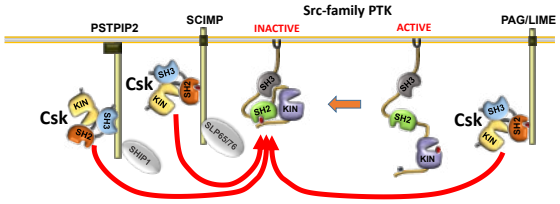

Fig. 1. Schematic representation of the regulation of Src-family kinases by Csk-binding proteins. Csk is recruited to the proximity of Src-family kinases by membrane adaptors such as SCIMP, PSTPIP2, PAG or LIME and then phosphorylates negative regulatory tyrosine at the C-terminus of Src-family kinases. This phosphorylation facilitates transition of Src-family kinases to the closed inactive conformation.

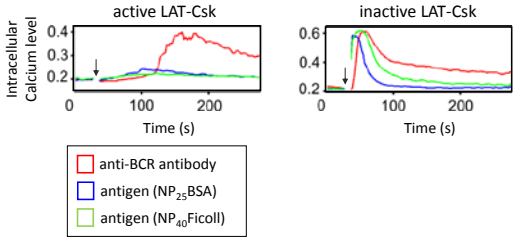

Fig. 2. One of the early outcomes of B-cell antigen receptor (BCR) stimulation is a rapid increase in intracellular calcium concentration. This image shows the effect of forced membrane targeting of Csk on this type of calcium response. Csk was targeted directly to the plasma membrane via fusion with the extracellular and transmembrane domain of transmembrane adaptor protein LAT (LAT-Csk). The left panel shows strongly reduced and delayed calcium response in the presence of membrane-targeted Csk (compare to the right panel where LAT-Csk activity was abolished by point mutation in the Csk kinase domain). The arrow points to the time when the stimulus was added to the sample.

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Not in the picture: Klára Kotlabová, BSc / diploma student (until 2013), István Dányi, MSc / PhD Student (until 2013)

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. Our current work focuses on understanding the modulation of MT properties by signal transduction molecules, the function of γ-tubulin forms, and on molecular and functional characterization of the regulators of MT nucleation. To address these questions, the techniques of molecular biology, biochemistry and immunology are being used, as well as a variety of microscopic techniques, including TIRF microscopy, SIM, live cell imaging and quantification of MT plus-end dynamics. Our results demonstrate that p21-activated kinase interacting exchange factor (βPIX) and G protein-coupled receptor kinase-interacting protein 1 (GIT1) play an important role in MT nucleation. Both proteins can associate with centrosomes of interphase cells. Microtubule regrowth and phenotypic rescue experiments showed that βPIX and GIT1 represent, respectively, negative and positive regulators of MT nucleation. Moreover, in mast cells MT nucleation is modulated by Ca2+, which affects γ-tubulin binding properties. We have also shown that both human γ-tubulins are nucleation competent but differ in their properties and expression. Accumulation of γ-tubulin 2 in mature neurons, in the face of predominant γ-tubulin 1 expression in these cells, may reflect additional γ-tubulin 2 function(s) in the neurons. We have demonstrated that ectopic expressions of γ-tubulin complex proteins GCP2 and GCP3 may represent novel markers in the pathobiology of glioblastoma multiforme, the most common and deadliest form of primary brain cancers. Although GCP2 and GCP3 are assumed to be typical cytosolic proteins, they are, similarly as γ-tubulin, also present in nucleoli of glioblastoma cells. Finally, we have introduced new methods for quantification of α-tubulin isotypes and for detection of tau proteins in cerebrospinal fluids.

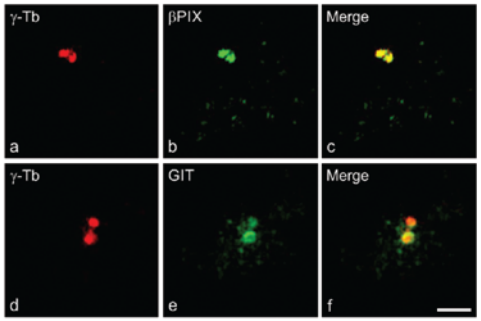

Fig. 1. Subcellular localization of GFP-tagged βPIX and GIT1 in bone-marrow mast cells Cells expressing TagRFP-γ-tubulin and βPIX-GFP or GIT1-GFP were fixed and evaluated in centrosomal region by super-resolution microscopy. (a-c) Localization of γ-tubulin (a) and βPIX (b). Superposition of images (c, γ-tubulin, red; βPIX, green). (d-f) Localization of γ-tubulin (d) and GIT1 (e). Superposition of images (f, γ-tubulin, red; GIT1, green). Bar, 2 μm.

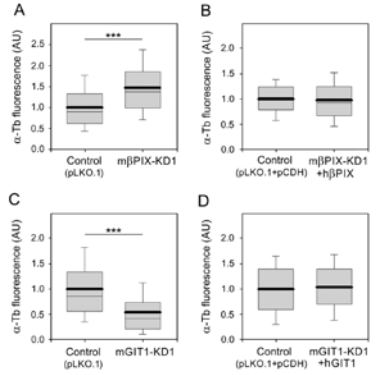

Fig. 2. βPIX and GIT1 proteins affect microtubule nucleation in bone-marrow mast cells The distributions of α-tubulin fluorescence intensities (arbitrary units, AU) in 1 μm ROI at 1.5 min of microtubule regrowth shown as box plot diagrams. (A) βPIX-depleted cells (mβPIX-KD1) relative to control cells (Control, pLKO.1). (B) βPIX-depleted cells rescued by hβPIX (mβPIX-KD1 + hβPIX) relative to control cells (Control, pLKO.1 +pCDH). (C) GIT1-depleted cells (mGIT1-KD1) relative to control cells (Control, pLKO.1). (D) GIT1-depleted cells rescued by hGIT1 (mGIT1-KD1 + hGIT1) relative to control cells (Control, pLKO.1 + pCDH).

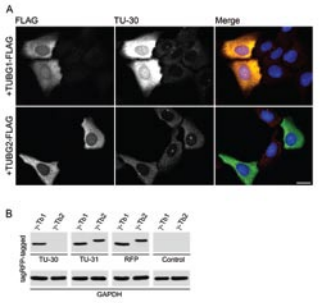

Fig. 3. Discrimination of human γ-tubulins Human γ-tubulin 1 is specifically recognized by anti-peptide antibody TU-30. (A) U2OS cells expressing human FLAG-tagged γ-tubulin 1 (TUBG1-FLAG) or γ-tubulin 2 (TUBG2-FLAG) were immunofluorescence stained for FLAG (green) and γ-tubulin 1 (red; TU-30). DNA was labelled with DAPI (blue). Scale bar, 20 μm. (B) Immunoblots of total cell lysates from cells expressing TagRFP-tagged human γ-tubulin 1 (γ-Tb1) or γ-tubulin 2 (γ-Tb2), probed with mouse antibodies to γ-tubulin (TU-30 and TU-31), tagRFP (RFP) or GAPDH. In control samples, only secondary anti-mouse antibody was applied.

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- ASCR, M200521203 – Microtubule regulatory proteins: novel target molecules for the therapy of brain cancers, 2012-2015, P. Dráber
- MH, NT14467 – Microtubule regulatory proteins as new biomarkers of gliomas, 2013-2015, P. Dráber
- MEYS, LD13015 LD-COST – Microtubules in activated mast cells – targets for innovative therapies, 2013-2014, P. Dráber
- GACR, GPP302/11/P709 – Analysis of microtubular changes during activation of mast cells, 2011-2013, V. Sulimenko
- TACR, TA01010436 – New generations of DNA aptamers, 2011-2013, Pe. Dráber, Pa. Dráber

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Not in the picture: Zuzana Hájková, MSc / PhD Student, Věra Vosecká, MSc / Research Assistant (maternity leave)

Laboratory of Signal Transduction

Plasma membrane signalosomes, immunoreceptor signalling, KIT and tetraspanin activation, chemotaxis

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Our long-term research goal is to understand the molecular mechanisms governing signal transduction from the plasma membrane receptors to the cytoplasm. We focus on the high-affinity IgE receptor, KIT, and tetraspanins in mast cell degranulation and chemotaxis. We also analyse the role of transmembrane adaptor proteins (NTAL, LAT, and PAG), galectins, and endoplasmic reticulum-associated proteins (STIM1, ORMDL3) and cross-talk of all these proteins during cell activation. To reach our goal, techniques of molecular biology, immunology, immunochemistry and immunohistochemistry are used. The techniques include use of mice with genetically enhanced or reduced expression of the proteins studied (through lentiviral transduction of proper probes or CRISPR/Cas techniques), expression profiling, and high-throughput screenings. Our research also involves development of new unique antibodies and DNA aptamer probes, as well as a variety of microscopic techniques (live cell imaging, TIRF microscopy, and super-resolution microscopy). An integral part of our studies is to verify performance of the signalling pathways under in vivo conditions. To this end we established several systems for analysis of mast cell activation, including passive cutaneous anaphylaxis and passive systemic anaphylaxis measured by computerized telemetry. Using these and other techniques we found and described new functions of the transmembrane adaptor proteins, tetraspanins, ORMDL3 protein, and galectin 3. Our studies deepen knowledge of the cellular and molecular mechanisms of the cells involved in allergic and inflammatory diseases, a prerequisite for development of anti-allergic and anti-inflammatory drugs.

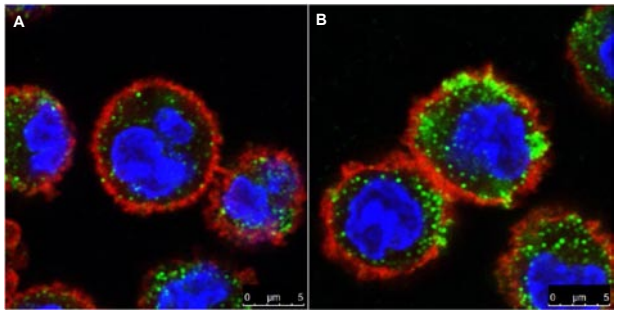

Fig. 1. Confocal microscopy images of IgE-sensitized bone marrow-derived mast cells before activation (A) and 5 min after activation with antigen (B). In activated cells, elevated amount of plasma membrane-bound signalosomes with tyrosine phosphorylated proteins is observed. Red – high-affinity IgE receptor with bound IgE detected with anti-IgE conjugated to Alexa Fluor 568. Green – tyrosine phosphorylated proteins detected with phosphotyrosine-specific antibody, followed by anti-IgG-Alexa Fluor 488 conjugate. Blue – nucleus stained with Hoechst 33258 Stain.

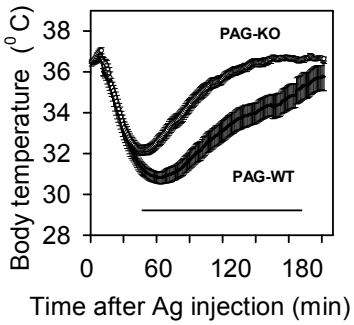

Fig. 2. The figure shows changes in body temperature in wild-type (WT) and PAG-deficient (PAG-KO) mice during passive systemic anaphylaxis. The mice were passively sensitized with antigen-specific IgE and 24 h later challenged with antigen to induce systemic anaphylaxis. Body temperature responses at various time intervals after antigen administration were measured with an accuracy of ± 0.1°C using the VitalView data acquisition system with ER-4000 energizer receivers, G2 E-mitter transponders implanted intra-abdominally, and VitalView software (Mini Mitter). Means ± SE are shown. Statistically significant differences ($P<0.05$) between PAG-WT and PAG-KO mice are indicated by black line below the curves.

- TACR, TG01010066 – Applied molecular genetics and biology – IMG, sub-project: Hybrid DNA polymerase Twa with enhanced enzymatic activity, 2014-2016, Pe. Dráber
- GACR, 14-00703S – ORMDL family proteins in mast cell signalling, 2014-2016, P. Dráber
- GACR, 14-09807S – Signalling pathways involved in mast cell chemotaxis, 2014-2016, L. Dráberová
- GACR, GBP302/12/G101 – Molecular mechanisms of signalling through leukocyte receptors - their role in health and disease, 2012-2018, V. Hořejší, Pe. Dráber, D. Filipp
- MEYS, LD12073 - COST CZ – Membrane signalosomes of mast cells and basophils – targets for innovative therapies, 2012-2014, P. Dráber
- MIT, FR-TI3/067 – Genetically modified polymerases and their use for amplification of unpurified DNA, 2011-2014, Pe. Dráber
- GACR, GA301/09/1826 – Topography and function of Csk-binding proteins of the plasma membrane in mast cells, 2009-2013, Pe. Dráber
- GACR, GAP302/10/1759 – Function-structure relationships between transmembrane adaptor-based signalosomes in mast cells, 2010-2013, L. Dráberová
- TACR, TA01010436 – New generations of DNA aptamers, 2011-2013, Pe. Dráber, Pa. Dráber

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From the left: Pavol Utekal, MSc / Research Assistant, Petr Dráber, DSc /Head of Laboratory, Viktor Bugajev, PhD / Research Fellow, Ivana Hálová, PhD / Research Fellow, Tomáš Paulenda, MSc / PhD Student, Romana Budovičová, MD / Research Assistant, Monika Bambousková, MSc / PhD Student, Magda Tůmová, PhD / Postdoctoral Fellow (maternity leave until IX/2014), Lucie Stegurová, MSc / PhD Student, Sergi Junyent Espinosa, Oleksii Redchenko, PhD / Research Fellow, Iva Polakovičová, MSc / PhD Student, Hana Mrázová / Technician, Lubica Dráberová, PhD / Research Fellow, MSc / PhD Student (currently abroad)

Not in the picture: Helena Dráberová

Laboratory of Immunobiology

Immune tolerance, TLRs in embryonic haematopoiesis, TCR signalling

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The main goal of our research is to elucidate the mechanism(s) guiding the process of central and peripheral tolerance. In the last couple of years, our research refocused on the contribution of cellular and molecular factors controlling the process of central and chiefly peripheral tolerance. We have shown that the physiological role of enteric α-defensin production in the thymus is critical for the maintenance of central tolerance in the small intestine. These molecules, expressed by Paneth cells in the crypts of small intestine, are also expressed by a sizable fraction of medullary thymic epithelial cells (mTECs), 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 who are deficient in AIRE function. Moreover, our new mouse model of APECED demonstrated that self-reactive enteric α-defensin-recognizing T cells alone are sufficient to drive the process of initiation of Paneth cell destruction (Fig. 1), leading to intestinal microbiome dysregulation and enhanced Th17 responses, which further amplify inflammatory autoimmunity in the intestine. In addition, we have characterized a functionally distinct lymph node cell population with the capacity to delete self-reactive CD8+ and CD4+ T cells or mediate conversion of the latter into Tregs (manuscript in preparation).

We are also very interested in the expression pattern and function of Toll-like receptors (TLRs) and other TIR domain-containing immune-related proteins during the early mammalian embryogenesis (Fig. 2). We have shown that TLRs expressed on embryonic macrophages couple inflammatory signals to iron metabolism during early ontogenesis. In addition, Toll-like receptor 2 (TLR2) seems to be a suitable surface marker that allows tracking the earliest haematopoietic progenitors in a precirculation embryo. Our newly generated transgenic mice, which enable performing genetic lineage tracing experiments, provided evidence that these early TLR2 expressing progenitors contribute not only to primitive but also to definitive haematopoiesis (manuscript submitted).

We also continue in our effort to understand the earliest biochemical events leading to the activation of T cells. This mainly concerns the processes associated with the regulation of the proximal T-cell signalling where two Src-family tyrosine kinases (SFK), Lck and Fyn, provide critical functions. Towards this end we have identified several candidate proteins involved in the regulation of translocation of Lck to lipid rafts via linking this process to microtubular cytoskeletal network (manuscript submitted).

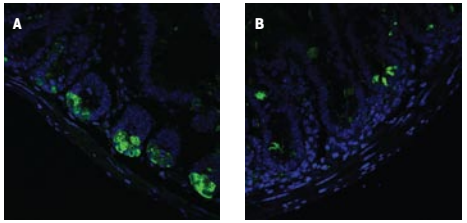

Fig. 1. The loss of Paneth cells visualized by lysozyme staining (green) on small intestinal sections in the absence (A) and presence (B) of enteric defensin-specific self-reactive T cells.

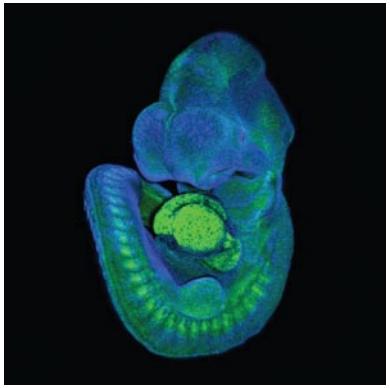

Fig. 2. A macro confocal macroscopy image of a mouse E10.5 embryo stained with Hoechst 33342 (blue), and paternally inherited EGFP fluorescence (green) driven from the β-actin promoter which enables distinguishing haematopoietic cells of maternal and embryonic origin .

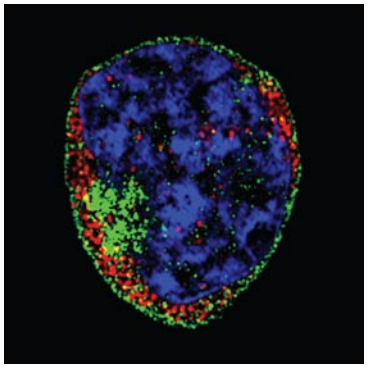

Fig. 3. Jurkat T-cell stained with anti-Lck antibody (green) and antibody against the candidate Lck-interacting protein (red) and visualized by N-SIM super-resolution microscope.

- TACR, GAMA TG01010066 – Applied molecular genetics and biology – IMG, 2014-2016, D. Filipp
- GACR, GBP302/12/G101 – Molecular mechanisms of signalling through leukocyte receptors, their role in health and disease, 2012-2018, V. Hořejší, Pe. Dráber, D. Filipp
- GACR, 14-17194P – Synthetic oligofuranosides, their enzymatic preparation and the mode of cellular signalling, 2014-2016, I. Chlubnová
- GACR, GA310/09/2084 – Characterization of the molecular machinery regulating the recruitment of signalling molecules to lipid rafts, 2009-2013, D. Filipp

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From the left: Adéla Fellnerová / Diploma Student (since October 2014, Dominik Filipp, PhD / Head of Laboratory, Jana Balounová, MSc / PhD Student, Research Assistant (since July 2014), Tomáš Brabec, BSc (since October 2014), Martina Benešová, MSc / Research Assistant, Matouš Vobořil / Diploma Student, PhD Student (since September 2014), Jan Dobeš, MSc / PhD Student, Ondřej Ballek, MSc / PhD Student, Lenka Súkeníková, BSc (since October 2014), Iva Šplíchalová / PhD Student (since September 2014)

Not in the picture: Aleš Neuwirth, MSc / PhD Student (until January 2013), Vijay Bharathi Arumugham, MD / PhD Student (until March 2013), Ilona Chlubnová / Postdoc (since January 2014)

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" *Hst1f* 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. The second hybrid sterility gene, *Hstx2*, showing Dobzhansky-Muller incompatibility with *Prdm9*, was mapped to a *4.7 Mb* interval on Chromosome X. Six protein-coding genes and a cluster of miRNA genes are tested as possible candidates of *Hstx2*. To characterize the incompatibilities underlying hybrid sterility, we phenotyped reproductive and meiotic markers in male mice 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, constructed in our laboratory are used for dissecting the genomic architecture of sterility of *Mus m. musculus* x *Mus m. domesticus* hybrids. We study meiotic X-chromosome inactivation by genome-wide expression profiling and by monitoring the 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.

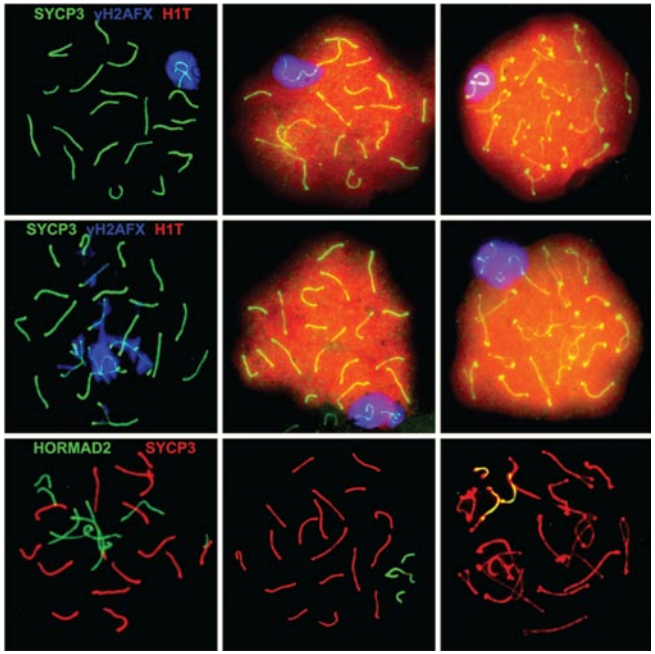

Fig. 1. Synaptic failure of homologous chromosomes in PWD x B6 inter-subspecific hybrids. SYCP3 protein component of synaptonemal complexes, phosphorylated histone H2AFX, testis-specific histone H1T and HORMAD 2 protein decorated unsynapsed chromosome axes are visualized by immunostaining of chromosome spreads of pachytene and diplotene primary spermatocytes. First row – fertile control B6 parent. Second and third row – sterile (PWD x B6) F1 hybrid. Note the disrupted sex body carrying X and Y chromosomes in early-mid pachytene of sterile males (first column).

- GACR, GA13-08078S – Genomic architecture and molecular basis of hybrid sterility of the mouse, 2013-2017, J. Forejt
- GACR, 14-20728S – Subspecies-specific function of meiotic genes in mouse gametogenesis, 2014-2016, O. Mihola

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Standing from the left: Ondřej Mihola, PhD / Research Associate, Václav Gergelits / PhD Student (until 2014), Petr Jansa, PhD / Research Fellow, Petr Flachs, MSc / PhD Student, Vladana Fotopulosová, MSc / Research Assistant, Prof. Jiří Forejt, MD, DSc / Head of Laboratory
Sitting from the left: Mária Balcová (Dzúr-Gejdošová) MSc / PhD Student, Barbora Faltusová, M.Sc / PhD Student, Irena Chvátalová, MSc / Research Assistant, Jana Perlová / Technician, Soňa Gregorová, MSc / Research Assistant, Lenka Kašíková / Diploma student (since 2013)

Not in the picture: Tanmoy Bhattacharyya, MSc / PhD Student, Lenka Šebestová / Diploma student (since 2014)

Laboratory of Viral and Cellular Genetics

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

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Retrovirus replication cycle starts by specific binding of retroviral envelope proteins to host cell receptors. After entering host cells, retroviruses integrate into the host chromosomes, and use the cell transcription and proteosynthesis machineries to express retroviral proteins and propagate their own progeny. At multiple levels, cellular restriction factors regulate retroviral replication. 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. Avian leukosis virus subgroup J (ALV-J), an important pathogen of domestic poultry, infects chickens and turkeys, whereas other galliform species are resistant thanks to a single amino-acid substitution in cell surface Na+/H+ exchanger (NHE1), the receptor for the virus. We now screen the NHE1 receptor in domestic chickens and wild ducks in order to find genetic sources for the resistance and predict the spread of ALV-J in natural reservoirs. 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 we developed strategies of protection from DNA methyltransferases by simple modifications of retroviral regulatory sequences. Now, our main effort is focused on the epigenomics of retroviral integration sites. The epigenomic approach to retrovirus integration reveals that transcriptionally active proviruses are preferentially localized close to the transcription starts of targeted genes or in enhancer regions. The protective region around the transcription start site is marked by enrichment in H3K4 trimethylation, demethylation, and H3K9 acetylation. Retrovirus restriction by the host cell is particularly effective in cross-species transmission. In a classic model of rodent cells transformed with Rous sarcoma virus, we studied the molecular mechanisms of virus rescue. Fusion with chicken cells permissive for Rous sarcoma virus provides the rodent cells with factors required for proper splicing and transport of viral RNA. Last but not least, our laboratory deals with endogenous retroviruses. We studied the epigenetic control and the possible role of hydroxymethyl cytosine in expression of syncytin-1, a fusogenic envelope glycoprotein encoded by human endogenous retrovirus ERVWE1. We are interested in the process of endogenization displayed in a model of polymorphic and infectious endogenous retrovirus in the mule deer genome. Using a new two-step computational screen, we discovered a new endogenous retrovirus in the genome of Malayan colugo. This is the first endogenous lentivirus identified in the Euarchonta lineage, which includes primates, and represents the oldest member of the lentivirus genus.

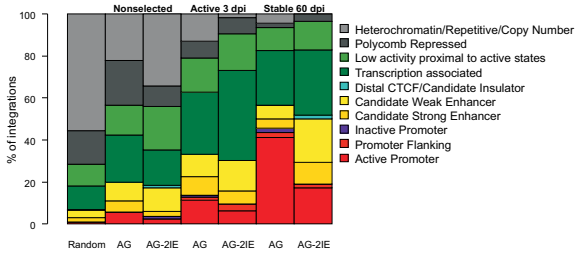

Fig. 1. An example of retrovirus integration analysis. In contrast to random distribution, retroviruses are overrepresented in certain chromatin segments. Selection for transcriptional activity favours retroviruses integrated into active promoter and enhancer regions.

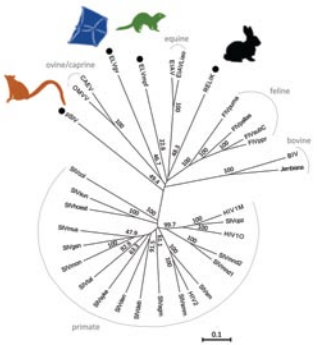

Fig. 2. Phylogenetic relationship of new endogenous retrovirus ELVgv to other lentiviruses. Endogenous lentiviruses described so far in rabbit, ferret, grey mouse lemur, and colugo are denoted.

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- MA, QJ1210041 – New type of vaccine against chicken viral diseases, 2012-2016, J. Hejnar
- MEYS, LK11215 LK-NÁVRAT – Regulation of active endogenous retroviruses in the mammalian genome, 2012-2016, D. Elleder
- MH, NT14601 – Syncytins as markers of germ line tumours, 2013-2015, J. Hejnar
- GACR, GA13-37600S – Expression of fusogenic human endogenous retroviruses in germ line tumours, 2013-2015, J. Hejnar
- GACR, GA13-30983S – Chicken polymorphisms in retroviral receptors and their potential for cross-species transmission, 2013-2015, J. Plachý
- GACR, 14-34873S – Epigenomics of retroviral integration, 2014-2016, J. Hejnar
- GACR, 14-32547S – Sensing of HIV-1 by plasmacytoid dendritic cells: dichotomy of immunoreceptor signalling, 2014-2017, I. Hirsch
- GACR, GAP502/11/2207 – Avian sarcoma and leukosis virus-based vectors and their potential for transgenesis in chicken, 2011-2013, J. Hejnar

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From the left: Markéta Reinišová, MSc / Research Assistant, Martina Benešová / PhD Student, Kateřina Trejbalová, PhD / Research Associate, Jan Vlasatý / Bachelor Student, Ivan Hirsch, PhD / Research Fellow, Kryštof Štafl / Diploma Student, Josef Geryk, PhD / Research Fellow, Helena Fábryová, MSc / PhD Student, Jiří Plachý, PhD / Research Fellow, Magda Matoušková, PhD / Postdoctoral Fellow, Dalibor Miklík, MSc / PhD Student, Tomáš Hron, MSc / PhD Student, Volodymyr Stepanets, PhD / Postdoctoral Fellow, Dana Kučerová, MSc / Research Assistant, Jiří Hejnar, PhD / Head of Laboratory, Albert Font Haro, MSc / PhD student, Anna Lounková, MSc / PhD Student, Martina Slavková / Diploma Student, Daniel Elleder, PhD / Research Associate, Denisa Kovářová, MSc / PhD Student.

Not in the picture: Prof Jan Svoboda, DSc / Research Fellow, Filip Šenigl, PhD / Research Associate, Lenka Mikušová / Technician.

Laboratory of Molecular Immunology

Membrane microdomains, chimeric antigen receptors, myeloid leukaemia, C/EBP transcription factors

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In 2013-2014 our laboratory was dealing with three topics:

1. Membrane rafts and immunoreceptor signalling (principal investigator Václav Hořejší).

For many years a major topic of our laboratory has been signalling molecules present in membrane rafts, namely several transmembrane adaptor proteins discovered previously by us, and their involvement in immunoreceptor signalling. Currently we are working on clarification of the relationship between various types of native membrane microdomains and detergent-resistant membrane fragments (DRMs).

2. Chimeric antigen receptors (CARs) (principal investigator Pavel Otáhal).

CARs are transmembrane constructs expressed in T lymfocytes capable of (a) specific recognition of e.g. tumour antigens and (b) effective signalling resulting in killing of the recognized tumour cell. CARs appear to be promising tools for cancer immunotherapy. We are currently developing new types of such potentially clinically useful CARs.

3. Mechanisms of leukemogenesis (principal investigator Meritxell Alberich-Jorda).

Acute myeloid leukaemia (AML) is a malignant haematopoietic disease that represents over 90% of acute leukaemias in adults. Changes in expression of critical transcription factors have been shown to deregulate haematopoiesis and aberrations in myeloid transcription factors have been observed in AML patients. Our research team is particularly interested in the CCAAT/enhancer binding protein (C/EBP) transcription factor family, which regulates the commitment of haematopoietic stem cells towards the myeloid lineage. Specifically, we investigate the functions of C/EBPγ and C/EBPα transcription factors and their target genes in normal haematopoiesis and malignant transformation in AML. Also, we aim to identify small molecules able to reactivate targets of the C/EBPα, ultimately resulting in therapeutic restoration of granulocytic differentiation in AML.

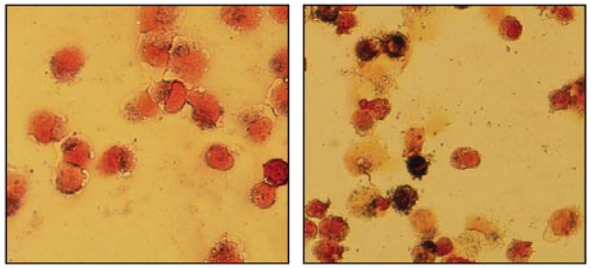

Fig. 1. K562 cells were transfected with constructs encoding transcription factor C/EBPα and oestradiol receptor (ER). Upon β-oestradiol stimulation C/EBPα-ER translocates to the nucleus and induces granulocytic differentiation, as demonstrated by the blue signal upon Nitroblue tetrazolium staining (right). Controls expressing only ER but not C/EBPα do not differentiate (left).

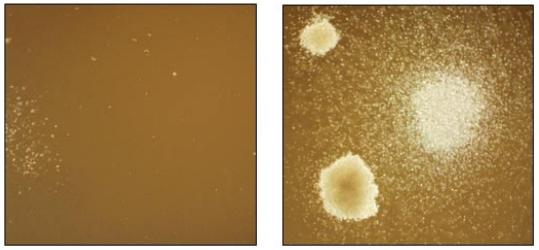

Fig. 2. Expression of dominant-negative transcription factor TCF4 (dnTCF4) in murine bone marrow cells strongly enhances their differentiation into colonies of myeloid and lymphoid cells in vitro (right), as compared to controls (left).

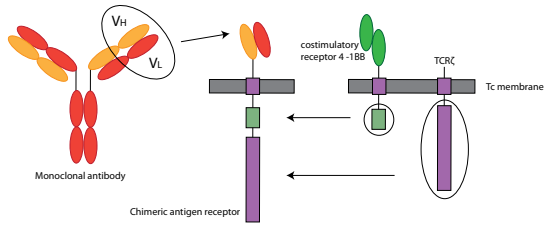

Fig. 3. Schematic view of construction of a chimeric antigen receptor composed of antigen recognition domain (originating from a mAb) and signalling domains taken from 4-1BB (CD137) and TCR ζ .

- GACR, GBP302/12/G101 – Molecular mechanisms of signalling through leukocyte receptors, their role in health and disease, 2012-2018, V. Hořejší, Pe. Dráber, D. Filipp
- MH, NT13462 – Optimization of immunotherapy and flow cytometric minimal residual disease assessment in resistant acute lymphoblastic leukaemia, 2012-2015, P. Otáhal
- MEYS, LK21307 LK-NÁVRAT – C/EBPγ in normal haematopoiesis and acute myeloid leukaemia: identification of molecular mechanisms involved in cell transformation, 2013-2015, M. Alberich-Jorda

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From the left: Miroslava Kardošová, MSc / PhD Student, Prof. Václav Hořejší, PhD / Head of Laboratory, Meritxell Alberich-Jorda, PhD / Research Fellow, Pavla Angelisová, PhD / Research Fellow, Carlos Lozano Asencio/ Diploma Student, Jana Pokorná / Technician, Polina Zjablovskaja, MSc / PhD Student

Not in the picture: Eva Tvrzníková / Secretary, Pavel Otáhal, MD, PhD / Research Fellow, Barbora Svobodová, MSc / PhD Student, Šimon Borna / 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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Cell nucleus is a fascinating organelle, where some 6 x 109 base pairs of DNA fold as a nucleoprotein complex (i.e. chromatin) into higher-order arrays so as to fit in a structure measuring only 10 μm. The machineries for transcription of genes and processing of RNA products, for accurate DNA replication, repair and recombination are precisely regulated within the nucleus. Multiple protein-protein, protein-nucleic acid, and protein-lipid interactions take place in specific microenvironments forming functional domains. 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 in transcription and gene expression, (4) functions of nuclear lipids, (5) development of new microscopy methods for ultrastructural studies.

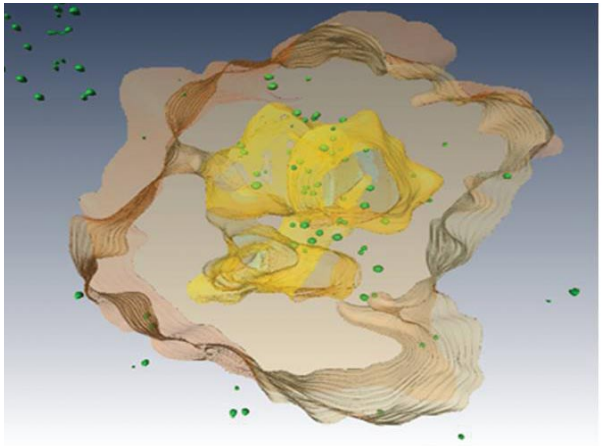

Fig. 1. PIP2 distribution in nucleolar subcompartments by TECNAI G2 20 LaB6 electron tomography. The fibrillar centre is pseudocoloured in yellow, the dense fibrillar component is in orange, and PIP2-containing areas are in green. (Yildirim et al., 2013)

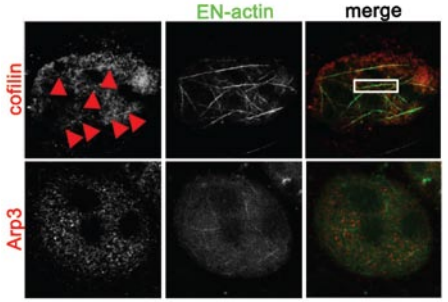

Fig. 2. Nuclear EN-actin (actin tagged with yellow fluorescent protein) filaments recruit Arp3 and cofilin. Co-localization of the nuclear EN-actin filaments with various actin-binding proteins was tested by indirect immunofluorescence microscopy in the U2OS cells. (Kalendova et al., 2014)

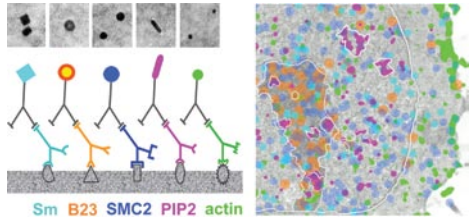

Fig. 3. Pathented method of simultaneous ultrastructural immunolabelling of five cellular antigens using metal nanoparticles of different shapes conjugated to secondary antibodies. The top panel: cubic palladium nanoparticles, silver-gold core-shell nanoparticles, 12-nm spherical gold nanoparticles, rod-like gold nanoparticles, 12-nm spherical gold nanoparticles. The right panel: mapping of labelled areas by respective antigens in the HeLa cell nucleus (Philimonenko et al., 2014)

- GACR, GAP305/11/2232 – Functions of myosin I and its binding partners in the cell nucleus, 2011-2015, P. Hozák
- MIT, FR-TI3/588 – Development of a kit for detection of mutations in structural proteins of a cell, 2011-2015, P. Hozák
- MIT, FR-TI4/660 – Multimodal holographic microscope, 2012-2014, P. Hozák
- TACR, TE01020118 – Electron microscopy, 2012-2019, P. Hozák
- MEYS, LD12063 LD-COST CZ – New nuclear functions of intermediate filaments, 2012-2014, P. Hozák
- MEYS, LH12143 LH-KONTAKT II – Cooperative contribution of actin- and myosin-families to the chromatin dynamics and tranion in the cell nucleus, 2012-2014, P. Hozák
- 1.07/2.3.00/30.0050 – Founding the expert platform for phenotyping and imaging technologies, 2013-2015, R. Sedláček, P. Hozák
- HFSP, RGP0017/2013 - Actin and actin-related proteins: probing their nuclear function, 2013-2016, P. Hozák
- FP7 EU, 262023 EURO-BIOIMAGING - Euro-BioImaging - Research infrastructure for imaging technologies in biological and biomedical sciences, 2010-2013, P. Hozák

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First row from the left: Margaryta Sobol, PhD / Research Fellow, Vlada Philimonenko, PhD / Research Fellow, Prof Pavel Hozák, DSc / Head of Laboratory, Jindřiška Fišerová, PhD / Research Fellow, Lenka Pišlová / Secretary, Karel Janoušek / Technician
Second row from the left: Jana Fukalová, MSc / PhD Student, Ilona Kalasová, MSc / PhD Student, Lukáš Pastorek MA, Zuzana Lubovská / Technician, Ivana Nováková / Technician, Anatoly Philimonenko, MSc / Research Assistant
Third row from the left: Tomáš Vacík, PhD / Research Fellow, Pavel Kříž / Technician, Lívia Uličná, MSc / PhD Student, Pavel Marášek, MSc / PhD Student, Robert Havalda, MSc / PhD Student, Iva Jelínková
Not in the picture: Jana Rohožková, PhD / Research Fellow, Alžběta Kalendová, MSc / PhD Student, Tomáš Venit, MSc / PhD Student, Lenka Jarolímová, MSc / Research Assistant, Markéta Morská, MA (EuroBioImaging) / Project Manager, M., Irina Studenyak / Research Assistant, Ondřej Šmíd, PhD / Research Fellow, Magdalena Skalníková, PhD / Research Fellow, Robert Havalda, MSc / PhD Student, Petr Martin / Diploma Student, Sukriye Yildirim, MSc / PhD Student

Laboratory of Cell and Developmental Biology

Colorectal cancer, liver, stem cells, TCF/LEF transcription factors, Wnt signalling

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The proper maintenance of adult tissues 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 elucidate the molecular mechanisms influencing the behaviour of normal and transformed intestinal and liver cells. Since the fate of these cells is determined by the so-called Wnt signalling pathway, our main focus is to identify genes activated by the Wnt pathway and/or encoding proteins directly involved in the intracellular signal transduction cascade.

The activity of the Wnt pathway undergoes complex regulation that ensures its proper functioning. The regulation may occur at several levels and includes both positive and negative feedback regulators. Recently, we characterized a negative feedback regulator of the Wnt signalling pathway, naked cuticle homologue 1 (Nkd1), in the intestine and liver and in tumours originating from these organs. We generated transgenic mice to trace Nkd1 expression in the gut and liver. Furthermore, we employed two mouse models of intestinal cancer to localize Nkd1 in tumour tissues. We also utilized an experimental collection of human sporadic tumours of the colon and liver to show that NKD1 can serve (along with three other genes involved in Wnt signalling) as a robust marker of neoplasia linked to aberrant Wnt signalling. Another important result in the current years was the identification of Troy as a novel modulator of Wnt signalling in stem cells of the intestinal epithelium. In addition, we identified monensin, a carboxylic polyether antibiotic, as a potent and specific inhibitor of the Wnt signalling pathway. The inhibitory effect of monensin on Wnt signalling was observed not only in various cell lines (including cells derived from human colonic carcinomas), but also in several in vivo tests such as the tail fin regeneration assay in zebrafish and body axis duplication assay in Xenopus. Importantly, monensin treatment significantly reduced the tumour burden in mice carrying multiple intestinal neoplasia. Since these mice represent an animal model of the human hereditary familial adenomatous polyposis (FAP) syndrome, our data imply that the antibiotic might be used as a chemopreventive agent for reduction of neoplastic growth in individuals suffering from the FAP syndrome.

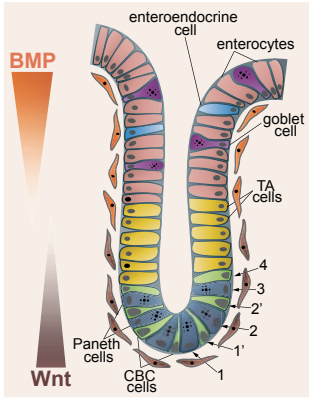

Fig. 1. Cellular architecture in the crypt of the small intestine
Intestinal homeostasis is sustained by crypt base columnar (CBC) stem cells that occupy the crypt floor in positions alternating with post-mitotic Paneth cells. The stem cells stochastically self-renew or give rise to committed daughter transit amplifying (TA) cells. As the progenitors further ascend the crypt, mesenchyme-derived BMP signalling promotes their differentiation towards predominant absorptive enterocytes, or secretory goblet and enteroendocrine cells that produce mucus and release peptide hormones, respectively. The pluripotency and proliferation of stem cells is maintained by Wnt cues, redundantly supplied by the stem-neighbouring Paneth cells and subepithelial myofibroblasts. Numbers assigned to individual cell positions in the crypt are indicated.

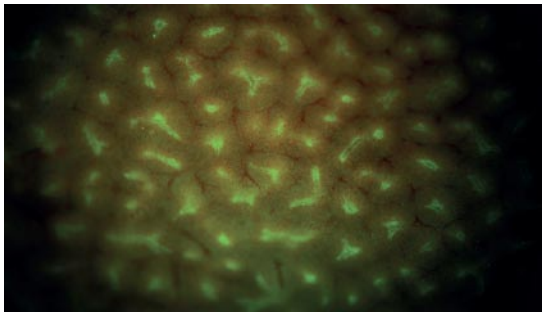

Fig. 2. Zonation of the liver visualized in Nkd1-CreERT2 transgenic mouse
Whole-mount fluorescent image of the Nkd1-CreERT2+/Rosa26R-EYFP liver taken four days upon tamoxifen administration. The perivenous hepatocytes are labelled by green fluorescence. Scale bar: 0.4 mm.

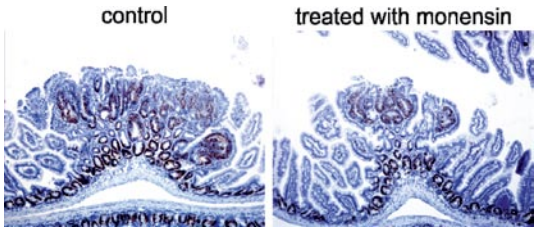

Fig. 3. Monensin treatment decreases the size of adenomas in mice
Haematoxylin- and anti-Ki67-stained sections of the jejunum of APC+/Min mice treated with monensin or vehicle alone (control).

- GACR, GAP304/11/1252 – Bacteria in aetiology, prevention and therapy of experimentally induced intestinal inflammation and colon cancer, 2011–2014, V. Kořínek
- GACR, GAP305/11/1780 – Wnt signalling in self-renewal and tumorigenesis of the intestinal epithelia, 2011–2014, V. Kořínek
- GACR, GAP305/12/2347 – Molecular mechanisms of the tumour suppressor function of the HIC1 gene, 2012–2015, V. Kořínek
- MEYS, EE2.3.30.0027 – Founding the Centre of Transgenic Technologies, 2012–2014, R. Sedláček, V. Kořínek, Z. Kozmík
- MIT, FR-TI4/802 – Development of new chemical compounds with anti-tumour activities or use in regenerative medicine, 2012–2015, P. Bartůněk, V. Kořínek
- GACR, 14-33952S – Molecular mechanisms underlying cell-fate decisions in the intestine, 2014–2016, V. Kořínek

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From the left: Eva Šloncová, MSc / Research Assistant, Kateřina Galušková, MSc / Research Assistant, Dušan Hrčkulák MSc / PhD Student, Oľga Babošová, MSc / PhD Student (since 2014), Vítězslav Kříž, PhD / Postdoc, Bohumil Fafílek, PhD / Postdoc, Vladimír Kořínek, PhD / Head of Laboratory, Martina Vojtěchová, PhD/ Research Fellow, Lucie Láníková, PhD / Postdoc, Nikol Baloghová/ Diploma Student, Lucie Janečková, PhD / Postdoc (since 2014), Monika Horázná, MSc / PhD Student

Not in the picture: Michaela Krausová, PhD/ Postdoc, Jiří Švec, MD, PhD / Postdoc, Jitka Stančíková, MSc / PhD Student (until 2014)

Laboratory of Transcriptional Regulation

Development and evolution, eye, brain, Pax genes, Wnt/β-catenin signalling

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We are interested in studies of 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 the Pax gene family. We use a combination of gain-of-function (transgenic) and loss-of-function (conditional knock-outs) approaches using laboratory mouse as a model organism to study embryonic development. We utilize several model systems including fish, amphioxus, *Platynereis* and cnidarians to study various aspects of evo-devo, especially the evolution of eyes and gene regulatory networks.

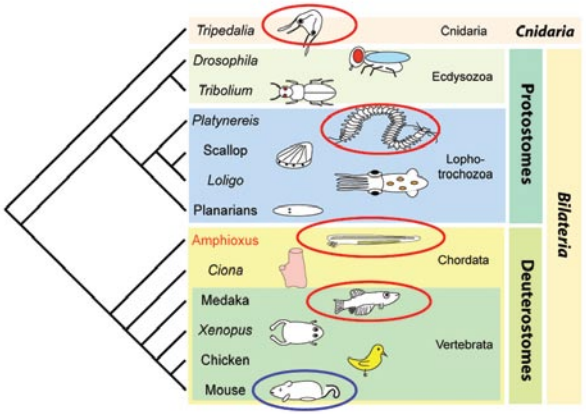

Fig. 1. Animal models used in the Laboratory for studies of development and evolution of development

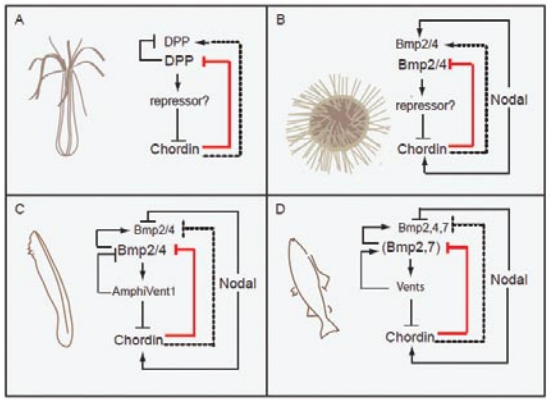

Fig. 2. Simplified schemes depicting Bmp-Chordin gene regulatory networks in cnidarian Nematostella (A), echinoderm sea urchin (B), cephalochordate amphioxus (C) and vertebrate zebrafish (D) during gastrulation. (Figure taken from Kozmikova et al., 2013)

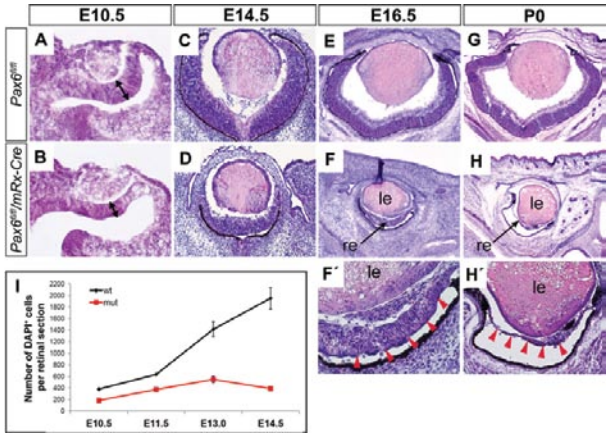

Fig. 3. Morphological consequences of Pax6 inactivation in early retinal progenitors. (Figure taken from Klimova and Kozmik, 2014)

- GACR, GAP305/10/2141 – Evolution of photoreceptors: insights from ancestral regulatory circuits, 2010-2014, Z. Kozmík
- GACR, GAP305/11/2198 – Genetics of mammalian eye development, 2011-2014, Z. Kozmik
- MEYS, EE2.3.30.0027 - Founding the Centre of Transgenic Technologies, 2012-2014, R. Sedláček, V. Kořínek, Z. Kozmík
- GACR, GAP305/12/2042 – The role of transcription factors Tcf in the induced cell pluripotency (iPS) and during neurogenesis, 2012-2015, O. Machoň
- MEYS, LK11214 LK-NÁVRAT – Genetic regulation of embryonic development of the brain and the eye, 2012-2016, O. Machoň
- MEYS, LH12047 LH-KONTAKT II – The role of alternative splicing in evolution of vertebrate body plan, 2012-2015, Z. Kozmik
- GACR, 14-20839P – The role of pituitary homeobox gene in anterior specification and left-right asymmetry in the basal chordate amphioxus, 2014-2016, V. Soukup
- ASCR, IAA500520908 – The role of Pax genes in eye evolution, 2009-2013, Z. Kozmik
- GACR, GCP305/10/J064 – Reconstructing urbilaterian photoreceptors: comparative study between Branchiostoma (Chordata) and Platynereis (Annelida), 2010-2013, Z. Kozmik

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Front row from the left: Jindra Pohořelá, MSc / Technician, Iva Dobiášovská / Diploma Student, Chrysoula Pantzartzi, PhD / Postdoctoral Fellow, Barbora Antošová, MSc / PhD Student, Veronika Nosková / Technician, Jitka Láchová, MSc / Technician
Back row from the left: Olga Tichá, MSc / PhD Student (since September 2013), Iryna Kozmiková, PhD/ Research Fellow, Andrea Kuželová, MSc / PhD Student (since September 2013), Lucie Klímová, MSc / PhD Student (now maternity leave), Zbyněk Kozmik, PhD / Head of Laboratory, Jiří Pergner, MSc / PhD Student, Peter Fabian, MSc / PhD Student, Vladimír Soukup, PhD / Postdoctoral Fellow

Not in the picture: Naoko Dupačová, PhD / Postdoctoral Fellow (maternity leave), Jana Smolíková, PhD / Postdoctoral Fellow (maternity leave), Kamil Matulka, PhD / Postdoctoral Fellow, , Anna Zitová, MSc / Technician (maternity leave), Michaela Liegertová, MSc / PhD Student, Jan Mašek, MSc / PhD Student, Radim Žídek, MSc / PhD Student, Ondřej Machoň, PhD / Research Fellow

Laboratory of Molecular Virology

Carcinogenesis, tumour microenvironment, metastasis, myofibroblast, photodynamic therapy, mitochondrial probes

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The research efforts of the group focus on the molecular mechanisms involved in several biological processes:

1) Initiation, promotion, and progression of experimental malignancies. In these studies insertional mutagenesis by MAV retroviruses is exploited to identify genes involved in the formation of kidney, liver and lung tumours in chicks.

2) Metastasis and fibrosis-related properties of tumour cells and myofibroblasts. Genes of the egr family serve as tools to study the metastatic potential of experimental tumours and the TGF-β signalling network in myofibroblasts.

3) Targeted intervention into pathological cell communication and induction of cell death in tumour cells with synthetic ligands and natural phospholipid derivatives. Various chemical compounds designed and synthesized by the cooperating group (Institute of Chemical Technology, Prague) are used as compartment-specific fluorescent dyes, 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 and egg yolk phospholipids with anti-tumour potential.

4) Replication and pathogenesis of avian retroviruses with extended host specificity. In this project novel retroviral genomes are constructed and their properties are analysed.

5) Fate determination in multipotent neural cells and differentiation of myogenic precursors. In these projects myb genes are used as molecular tools.

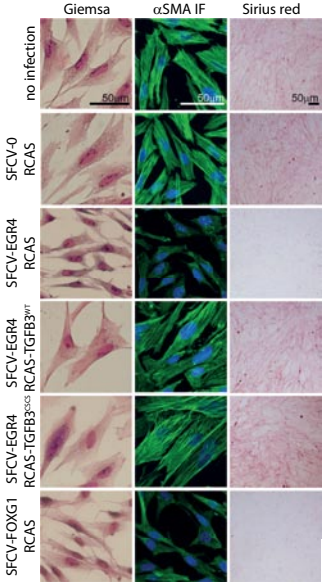

Fig. 1. Appearance and phenotypical features of cells with stable expression of EGR4 or FOXG1.
Cell morphology was visualized with Giemsa staining; αSMA (smooth muscle actin) immunofluorescence and Sirius red staining of deposited ECM (extracellular matrix) were performed to assess the myofibroblastic features of cells. Retroviruses used to infect the cells are indicated on the left. Cells constitutively expressing EGR4 or FOXG1 show loss of myofibroblast-typical prominent αSMA fibres and also a strong reduction in the ECM production. Concomitant overexpression of TGF-β3 restores the myofibroblastic phenotype in EGR4-expressing cells.

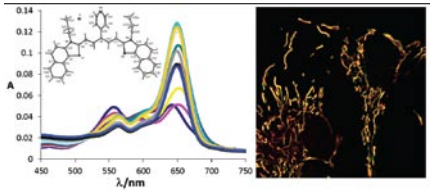

Fig. 2. Novel cell-permeable fluorescent probes for labelling and tracking mitochondria in living cells.
Fluorescent probes are based on symmetric γ-aryl substituted pentamethine salts displaying specificity for mitochondrial cardiolipin (left). The red staining pattern overlaps with staining of commercial MitoTracker Green in osteosarcoma U-2 OS cells (right).

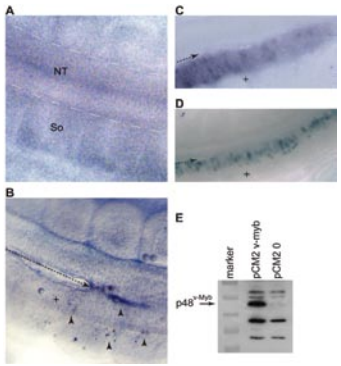

Fig. 3. Detection of Gremlin 2 mRNA-synthesizing cells in developing chicken embryos.
(A) The WISH (whole mount in situ hybridization) technique demonstrates the absence of Gremlin 2 mRNA in neural tissues of the truncal region in the HH stage 21 chicken embryo – the dorsal view (NT – neural tube, So – somite). (B) Detection of Gremlin 2 mRNA in chicken embryo electroporated with the v-myb expression vector (HH stage 11-12) and analysed 24 hours later – the dorsal view. Arrowheads point to a Gremlin 2-positive area and clusters of migrating cells. (C) WISH detection of Gremlin 2 mRNA in chicken embryo neural tube area (HH stage 11-12) electroporated with the gremlin 2 expression vector and analysed 24 hours later – the dorsal view. (D) WISH detection of v-myb mRNA following electroporation with v-myb vector. Experimental setting as in (C). (E) Western blot detection of the v-Myb protein in the v-myb electroporated side of the neural tube. In control experiments embryos were electroporated with the myb-minus vector (third lane). Dashed arrows in B, C, D represent needles used for the injection of expression vectors. The sign (+) in B, C, D marks the electroporated side of the neural tube.

- GACR, GAP303/11/1291 – New types of ligands interfering with cancer cell communication as a new therapeutic strategy, 2011-2014, J. Králová

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From the left: Petr Kašpar, PhD / Research Fellow, Jarmila Králová, PhD / Head of Laboratory, Michaela Bendová (Starostová), MSc / PhD Student, Marta Dvořáková, MSc / Research Assistant, Michal Dvořák, PhD / Research Fellow, Vladimír Pečenka, PhD / Research Associate, Vít Karafiát, MSc / Research Assistant, David Přikryl / Diploma Student

On the sceen: Jan Kosla, PhD / Postdoctoral Fellow, Petr Pajer, PhD / Postdoctoral Fellow, Mária Gašpareková/ Bachelor student

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. The majority of our data have been obtained using infection of *L. major*. Recently, we established the first genetic model of susceptibility to *L. tropica* and provided the first insight into the genetic architecture of susceptibility to this parasite. We have described eight loci on seven chromosomes and shown that the presence of individual symptoms of the disease is controlled by different subsets of the host's genes. The identification of the host's genes responsible for the specific symptoms of the disease induced by different *Leishmania* species will contribute to the understanding of the mechanisms of pathogenesis of leishmaniasis, similarly as comparative parasite genomics led to the identification of differentially distributed genes in *Leishmania* species inducing different pathology, and analysis of specific virulence factors revealed how different *Leishmania* species subvert or circumvent the host's defences. Such analysis will provide description of individual predisposition to specific symptoms of the disease and its probable course. Moreover, the possibility to compare genetics of the response to several *Leishmania* species will further help to understand the genetic basis of general and species-specific responses of the host. This will synergize with the future information on the genome sequence of *L. tropica* and interaction of its specific virulence factors with the immune system. Last but not the least: we have established a novel model for studying tick-borne encephalitis, the main tick-borne virus infection in Eurasia.

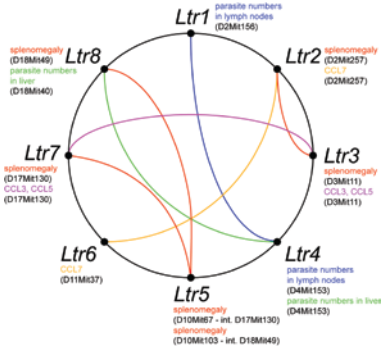


Fig. 1. Epistasis in control of susceptibility to *Leishmania tropica*
Phenotypes controlled by each locus are shown at their symbol in different colours. The coloured lines connecting the loci indicate interactions controlling the specific phenotypes.

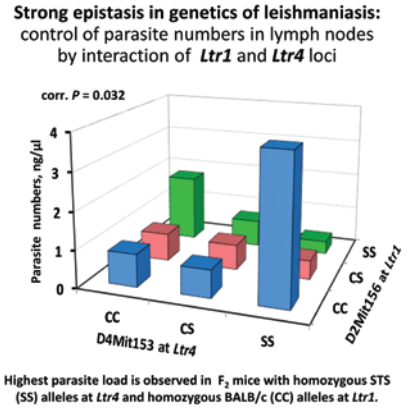
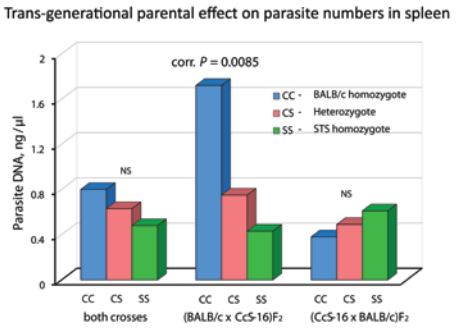


Fig. 2. Interacting loci *Ltr1* and *Ltr4*

Highest parasite load is observed in F_2 mice with homozygous STS (SS) alleles at *Ltr4* and homozygous BALB/c (CC) alleles at *Ltr1*.



Locus *Ltr3* linked to D3Mit25 influencing parasite numbers in spleen was significant only in the cross (BALB/c x CcS-16)F_2, but not in the cross (CcS-16 x BALB/c)F_2.

- GACR, GAP502/11/2116 – Differences in the clinical course of tick-borne encephalitis in the host and their genetic determination, 2011-2015, M. Lipoldová
- MEYS, LH12049 LH-KONTAKT – New genomic strategy for rapid identification of genes controlling development of infections and cancer, 2012-2015, M. Lipoldová
- GACR, GP13-41002P – Genetic control of parasite dissemination after *Leishmania* major infection, 2013-2015, T. Kobets
- GACR, 14-35944P – Analysis of interaction between *Leishmania* major and macrophages of susceptible and resistant mouse strains, 2014-2015, I. Grekov
- GACR, 14-30186S – Hidden relevant functional pathways in host response to *Leishmania* infection revealed in focused genomic constructions, 2014-2016, M. Lipoldová

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First row from the left: Tetyana Kobets, PhD / Postdoctoral Fellow, Tereza Pokorná, Bc/ Diploma Student (since September 2014), Marie Lipoldová, Assoc Prof, PhD / Head of Laboratory, V. Volkova
Second row from the left: Lucie Kocandová, MSc / PhD Student (since October 2014), Yahya Sohrabi, PhD / Postdoctoral Fellow, Matyáš Šíma, MSc / PhD Student

Not in the picture: Igor Grekov, PhD / Postdoctoral Fellow, Jarmila Vojtíšková, PhD / Research Fellow, Martina Slapničková, MSc / PhD Student, Karin Heyduková, Bc/ Diploma Student (since September 2014), Monika Buddeusová / Technician, Jan Bartůněk, Bc / Diploma Student (since September 2014), Valeriya Volkova, MSc / Research Assistant, Iva Kolářová, PhD / Postdoctoral Fellow (maternity leave), Marie Čepičková, MSc / PhD Student (maternity leave), Iryna Kurey, MSc / PhD Student (maternity leave)

Laboratory of Cancer Cell Biology

Cell cycle, checkpoint, DNA damage response, phosphorylation, oncogenic transformation

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Proliferation of cells is essential for keeping organisms alive and healthy and is accomplished by passing through interphase followed by nuclear division (mitosis) and cellular division (cytokinesis). In response to DNA damage, cells temporarily stop progression through the cell cycle (checkpoint) to prevent transmission of mutations to progeny. After completion of DNA repair, cells are allowed to re-enter the cell cycle (checkpoint recovery). Cells that are exposed to a massive genotoxic stress that exceeds the capacity of DNA repair are eliminated by programmed cell death. Radiotherapy and chemotherapy with genotoxic pharmaceuticals represent two commonly used non-surgical strategies in treatment of human tumours and they both rely on induction of cell death by genotoxic stress. Progression through the cell cycle and cellular responses to DNA damage are tightly controlled by interconnected signalling cascades. Malfunction of cellular checkpoints causes accumulation of mutations and can lead to genome instability, activation of oncogenes, and eventually to malignant transformation.

In our recently established laboratory we employ cell biology, molecular biology and biochemical approaches to identify the molecular mechanisms that control cellular responses to DNA damage. In particular, we focus on protein phosphatase PPM1D/Wip1 that plays an essential role in switching off the DNA damage response pathway, termination of the checkpoint and control of checkpoint recovery. PPM1D/Wip1 is an important negative regulator of the tumour suppressor p53. Recent data from transgenic mice and from human tumours implicate PPM1D/Wip1 as an oncogene. Our work aims to decipher the molecular mechanisms regulating the function of PPM1D/Wip1 in human cells and in mouse models. In addition, we use chemical genetics to evaluate PPM1D/Wip1 as a potential pharmacological target.

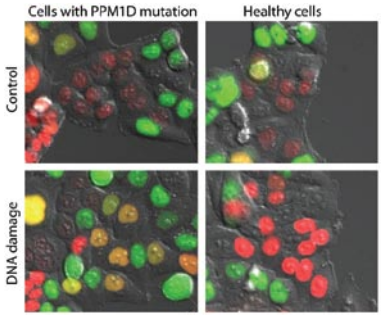

Fig. 1. Healthy cells do not proliferate in the presence of DNA damage and stop progression through the cell cycle in G1 phase (red). Cells carrying mutation in exon 6 of the PPM1D gene continue cell division despite the presence of DNA damage, progress to S phase and replicate their DNA (orange). Mutation in PPM1D does not affect cells in G2 (green). Courtesy of Indra A. Shaltiel and Libor Macurek

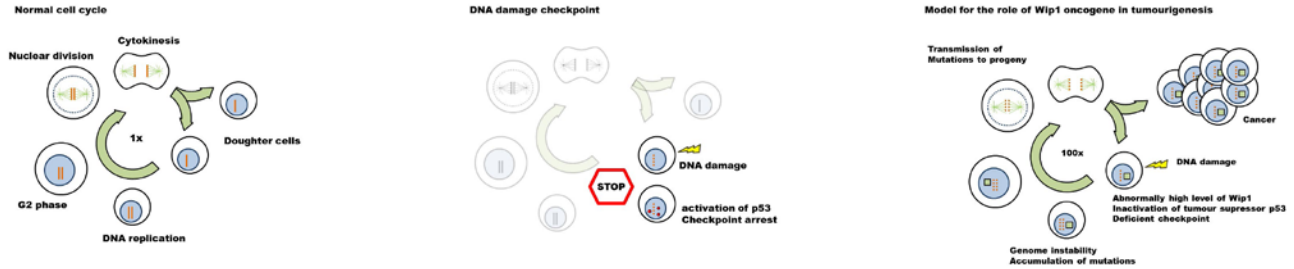

Fig. 2. Model for PPM1D mutation in cancer

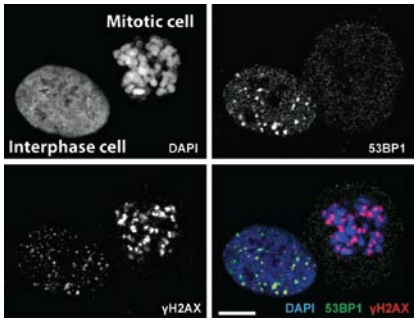

Fig. 3. After exposure to DNA damage, interphase cells form foci positive for 53BP1 and gH2AX. Mitotic cells form only gH2AX-positive foci but fail to recruit 53BP1 to the sites of DNA damage.

- GACR, GAP305/12/2485 – Structure and function of proteins involved in DNA damage signalling, 2012-2015, L. Macůrek
- GACR, GA13-18392S – Dynamics of DNA damage response in cells, 2013-2016, L. Macůrek
- GACR, 14-34264S – Role of R2TP complex in DNA damage response and cell proliferation, 2014-2016, Z. Líčeníková Hořejší
- MEYS, 7F14061 – Phosphorylation-mediated signalling in DNA damage response and cancer, 2014-2017, L. Macůrek
- Worldwide Cancer Research – Role of PPM1D/Wip1 truncating mutations in cancer predisposition, 2014, L. Macůrek

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From the left: Tomáš Liďák / Diploma Student, Soňa Pecháčková, MSc / PhD student, Jan Benada, MSc / PhD student, Andra Stefania Vieru, MSc/ PhD student (since October 2014), Libor Macůrek, MD, PhD / Head of Laboratory, Kamila Burdová, PhD / Postdoctoral fellow (since December 2014), Gabriela Jeníková, PhD (part time) / Postdoctoral fellow, Patrick von Morgen, MSc / PhD student (since October 2014), Monika Burócziová, PhD (part time) / Postdoctoral fellow

Not in the picture: Zuzana Líčeníková Hořejší, PhD / Research Fellow (since January 2014), Petra Kleiblová, MD, PhD (part time) / Postdoctoral fellow

Laboratory of Tumour Immunology

Anti-tumour immunotherapy, immunoediting, immunoepigenetics, cellular senescence

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As a long-term research programme we have been investigating the interactions between tumour cells and the immune system, with special attention paid to the mechanisms by which the tumour cells are capable to escape from immune responses. Our projects are also focused on the mechanisms of immunosuppression and its possible overcoming and, finally, on experimental anti-tumour immunotherapy and immunologic impacts of chemotherapy. Most of our studies employ murine models for tumours associated with human papilloma virus infection or for prostate cancer. MHC class I deficiency on tumour cells is a frequent mechanism by which tumour cells can escape from specific immune responses. We have found that epigenetic agents, namely DNA methyltransferase inhibitor 5-azacytidine, can induce expression of genes involved in the antigen-processing machinery and surface expression of MHC class I molecules on MHC class I-deficient tumour cells, which was associated with demethylation of the regulatory sequences of the corresponding genes. Activation of the genes encoding the components of the antigen-processing machinery is often mediated by interferon γ. We have demonstrated that gene expression mediated by this cytokine can be associated with DNA demethylation, suggesting that interferon γ can be considered as an epigenetic agent. Our next areas of interest are populations of immunoregulatory cells and their dynamics in the course of the tumour growth and therapy. We are namely interested in myeloid-derived suppressor cells, a cell population playing a critical role in mediating suppression of the anti-tumour immunity. We have found that 5-azacytidine can display both cytotoxic and differentiation effects on these cells. Our interest has also been turned to the field of cellular senescence induction in tumour cells upon treatment with genotoxic agents and to the role of cytokines in the senescence induction and maintenance. We believe that better understanding of the mechanisms by which senescent tumour cells can influence the tumour microenvironment and, on the other hand, whether and how the immune response can induce cellular stress and senescence in tumour cells, can bring clues important for our better insight into the tumour development, as well as for finding new therapeutic schemes. Besides our basic research programme we also perform contract research, mainly dealing with optimization of the dendritic cell-based immunotherapy combined with chemotherapy. Indeed, we are open for collaborations in the field of anti-tumour immuno- and chemotherapy, using our animal models and our immune response-monitoring expertise.

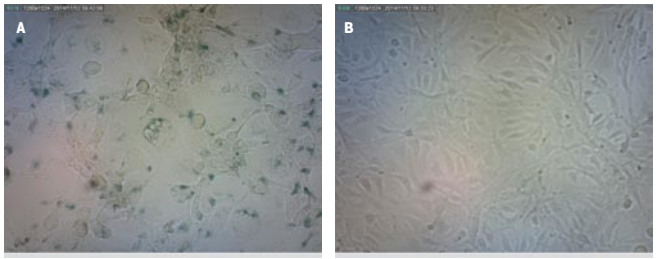
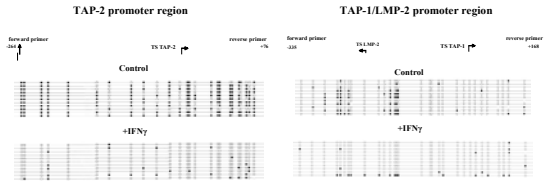


Fig. 2. Induction of cellular senescence in TC-1 tumour cells in response to docetaxel treatment (detected by β-galactosidase staining). Massive cellular senescence (blue stain) can be observed in docetaxel-treated cells (A) but not in control untreated cells (B).

Fig. 1. IFNγ-induced DNA demethylation of the TAP2 and TAP1/LMP2 promoters in TC-1/A9 cells analysed by bisulphite sequencing. DNA isolated from the IFNγ-treated and control untreated MHC class I-deficient TC-1/A9 cells was subjected to bisulphite conversion and cloned. Sequences from 11 clones from each sample are presented. After treatment with IFNγ, strong DNA demethylation of both the TAP2 and TAP1/LMP2 gene promoter regions was observed. White and black circles indicate unmethylated and methylated CpGs, respectively. Gene transcription start sites (TS) are indicated. This DNA demethylation was accompanied by increased MHC class I molecule expression on the cell surface. For details, see Vlkova et al., Oncotarget 2014.



- MH, NT14461 – Senescence cell elimination in minimal residual tumour disease therapy, 2013-2015, M. Reiniš
- GACR, 14-10100S – Utilization of novel mouse strains for investigation of the NK cell regulatory role in development and therapy of cancer, 2014-2016, M. Indrová
- GACR, GAP301/10/2174 – Epigenetic mechanisms in regulation of genes important for antigen presentation and antitumour immunity, 2010-2013, M. Reiniš
- GACR, GPP301/11/P220 – Mechanisms underlying cyclophosphamide-induced accumulation of myeloid derived suppressor cells, 2011-2013, R. Mikyšková

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From the left: Romana Mikyšková, MD, PhD / Research fellow, Jana Bieblová, MSc / Technician, Veronika Polláková, MSc, PhD / Postdoc, Milan Reiniš, PhD / Head of Laboratory, Marie Indrová, PhD / Research fellow, Georg Michlits (from 2014) / PhD student, Oleksander Korolov (from 2014) / PhD student, Renáta Turečková /Technician, Jana Šímová, PhD / Research fellow

Not in the picture: Ivan Štěpánek, Dipl. Ing., MSc, PhD / Postdoc, Lenka Fišerová (from 2014) / Undergraduate student, Michaela Horňáková / Undergraduate student, Magdalena Cebová (until 2013) / Undergraduate student, Veronika Hrušková (until 2014) / Undergraduate student, Zuzana Paračková (until 2013) / Undergraduate student, Lenka Fišerová (from 2014) / Undergraduate student

Laboratory of Structural Biology

Protein crystallography, HIV protease, human carbonic anhydrase IX, antibody engineering

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The main interests of our group are structural studies of various proteins of biological or medicinal interest using the method of protein crystallography. We use the structural knowledge to understand the protein function and in some projects also in modulating its function by design of specific inhibitors.
In our structure-based drug discovery project, we target enzymes from pathogenic organisms as well as human enzymes (e.g. human nucleotidases or cancer-specific carbonic anhydrase IX); the knowledge of protein structures provides a platform for the rational design of specific inhibitors.
Our group also focuses on engineering recombinant antibody fragments of potential diagnostic use. We employ several approaches aiming at practical use of recombinant antibody fragments.

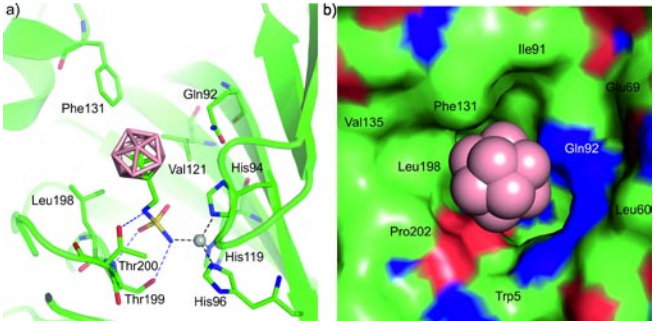

Fig. 1. Carborane-based inhibitors of human carbonic anhydrase IX were designed using a structure-based approach (reference 4).

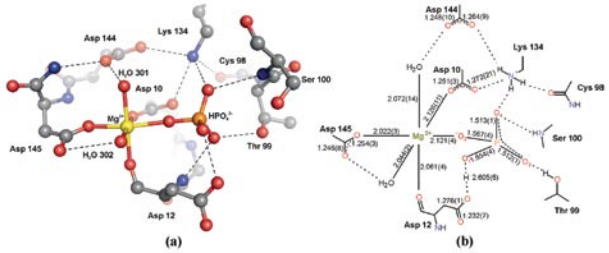

Fig. 2. High-resolution crystal structures of mitochondrial and cytosolic 5'-deoxyribonucleotidases with active site phosphate ions were used to estimate phosphate protonation and investigate differences in the active sites. These findings were applied to the design of a specific inhibitor (reference 2).

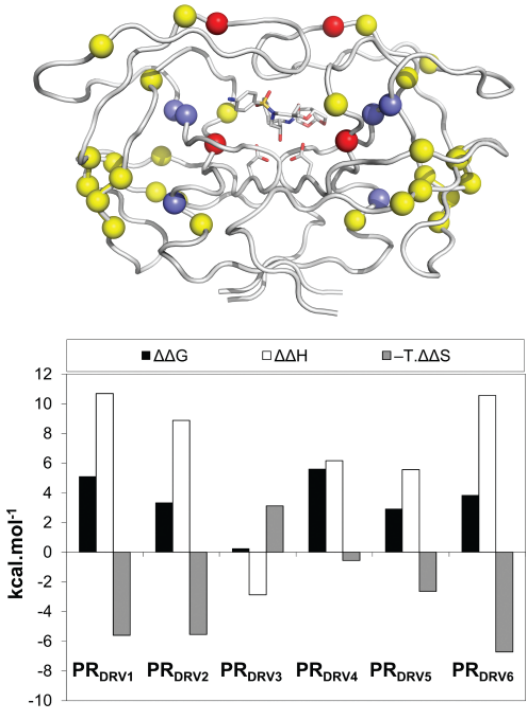

Fig. 3. Thermodynamic and structural analysis of HIV protease resistance to clinically used drug darunavir: analysis of heavily mutated patient-derived HIV-1 proteases (reference 3).

- TACR, TA02010797 – Labelling of recombinant antibody fragments with use of microfluidic systems, 2012-2014, J. Sedláček
- GACR, GA203/09/0820 – Structure-based drug design of specific nucleotidase inhibitors, potentially pharmacologically important compounds, 2009-2013, J. Brynda

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2. Pachl P, Fábry M, Rosenberg I, Simak O, Řezáčová P, Brynda J: Structures of human cytosolic and mitochondrial nucleotidases: implications for structure-based design of selective inhibitors. **Acta Crystallogr D Biol Crystallogr 2014** Feb;70(Pt 2):461-70.
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From the left standing: Petr Pachl, MSc / PhD Student, Petr Těšina, MSc / PhD Student, Juraj Sedláček, DSc / Research Fellow, Irena Sieglová, MSc / Research Assistant, Pavlína Maloy Řezáčová, PhD / Head of Laboratory, Assoc Prof Jiří Brynda, PhD / Research Fellow, Jitka Kredbová / Technician, Milan Fábry, PhD / Research Fellow
From the left sitting: Magdalena Hořejší, MSc / Research Assistant, Jana Škerlová, MSc/ PhD Student, Věra Mrkvičková / Technician, Vlastimil Král, PhD / Research Associate

Not in the picture: Veronika Krejčiříková, PhD / Postdoctoral Fellow (maternity leave)
Background: crystal structure of HIV protease in complex with a carborane inhibitor (PDB code 1ZTZ)

Laboratory of Transgenic Models of Diseases

Transgenesis, embryogenesis, proteases and their inhibitors, aging and epigenetics, neural development

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With an exceptional role in IMG, our department serves as an incubator in which research projects and groups of the BIOCEV project as well as national research infrastructure develop. Although thematically distinct, all groups, projects and activities 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). MMP and Klk proteases are partly responsible for controlling extracellular matrix-cell interactions affecting cell differentiation, survival, migration, and other processes. ADAM10 & ADAM17 proteinases 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 biological processes are to be initiated or terminated.

Ubiquitylation-mediated processes in health and disease. Using mutant mouse models we address the role of several ubiquitin ligases and deubiquitinases in mediating responses to environmental stressors. A main focus of these studies is to understand the role of ubiquitylation in regulating intestinal barrier function and to characterize links with human inflammatory bowel disease.

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.

Fig. 1. TALEN-assisted targeting of the ROSA locus: expression of turboRFP in founder mice was manifested by red colour of the skin. Photographs were taken at the age of 2 days (left) and 4 weeks (right). Strong expression of TurboRFP in founder mice was confirmed using fluorescent microscopy of tail biopsies (right). Expression of TagBFP in the flippase-positive embryo (left) derived from breeding of founder B7 with a flippase expressing mouse.

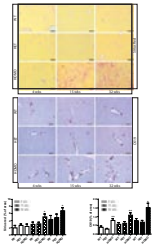

Fig. 2. Mutant mouse with liver-specific ablation of ADAM10 develops spontaneous fibrosis.

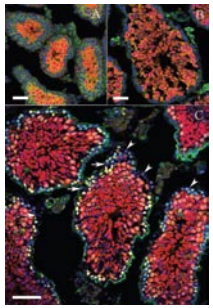

Fig. 3. Expression of EGFP protein in the testis of LRAT-EGFP reporter transgenic mouse from postnatal day 14 to adulthood. From postnatal day 14 to adulthood, EGFP protein (red) expression in the pLrat□GFP 17 reporter mouse is highly expressed in meiosis round spermatocytes (SCP3 positive) and in post meiosis spermatids (SCP3 negative), which are located more luminal.

- GACR, GAP303/10/2044 – The Impact of a liver-specific deficiency of growth factor sheddase ADAM10 on liver development and pathology, 2010-2013, R. Sedláček
- GACR, GAP305/10/2143 – Generation of mouse models for targeting stellate cells and myofibroblasts in the liver, 2010-2013, R. Sedláček
- GACR, GAP302/11/2048 – Function of metalloproteinases in colon epithelium and during development of experimental colitis and colon cancer, 2011-2014, R. Sedláček
- GACR, GA13-01710S – Reactivity of lung vessels in pulmonary hypertension, 2013-2017, K. Chalupský
- GACR, 14-33798P – Genomic instability and cardiovascular aging: the role of local and systemic mechanism, 2014-2016, M. Ďurík
- FP7 EU, 284501 INFRACOMP – Coordinating the cooperation of the ESFRI project Infrafrontier with the International Phenotyping Consortium (IMPC), 2011-2014, R. Sedláček
- FP7 EU, 312325 Infrafrontier-I3 – Development of mouse mutant resources for functional analyses of human diseases - Enhancing the translation of research into innovation, 2013-2016, R. Sedláček
- MEYS, EC OP CZ.1.07/2.3.00/20.0102 – Founding an expert team for the Centre for Phenogenomics, 2011-2014, R. Sedláček
- MEYS, EC OP CZ.1.07/2.3.00/30.0027 – Founding the Centre of Transgenic Technologies, 2012-2015, R. Sedláček, V. Kořínek, Z. Kozmík
- MEYS, EC OP CZ.1.07/2.3.00/30.0050 – Founding the expert platform for phenotyping and imaging technologies, 2012-2015, R. Sedláček, P. Hozák
- MEYS, LM2011032 INFRAFRONTIER-CZ – 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, 2012-2016, R. Sedláček
- MEYS, 7AMB13AT012 – Structural and functional analysis of interaction between calmodulin and plectin isoform 1a, 2013-2014, M. Gregor
- MEYS, LH14276 LH - KONTAKT II – Novel causative genes identification and functional study for selected Mendelian disorders, 2014-2016, R. Sedláček
- MH, IGA NT14451 – New technology for correction of mutations in monogenetic diseases by targeted reparation of mutations using specific nucleases, 2013-2015, R. Sedláček
- TACR, TA03011057 – Development of new methods to analyse environmental genotoxic stress and mutagenicity of potential pharmaceutical compounds, 2013-2016, R. Sedláček

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From the left:
Down: Makéta Pícková / Technician, Silvia Petrezselyová PhD / Postdoctoral Fellow, Lenka Sarnová MSc / Technician, Agnieszka Kubik-Zahorodna PhD / Research Associate, Olga Žbodáková MSc / Research Assistant, Zuzana Ileninová PhD / Postdoctoral Fellow, Dana Kopperová Msc / Technician, Monika Volčková MSc / Technician, Henrieta Pálešová MSc / Technician
Middle: Radislav Sedláček PhD / Head of Laboratory, Veronika Grešáková MSc / PhD Student, Shohag Bhattacharyya MSc / PhD Student, Björn Schuster PhD / Research Associate, Jana Šafránková B.A. / Project Manager, Veronika Libová MSc / Technician, Petr Kašpárek MSc / PhD Student, Attila Juhasz / Technician, Anna Laštůvková MSc / Technician, Inken M. Beck, Libor Daněk M.A. / Project Manager, Benoit Piavaux PhD / Postdoctoral Fellow
Top: Jolana Turečková PhD / Research Associate, Barbora Singerová MSc / Technician, Martin Gregor PhD / Group Leader, Karel Chalupský PhD / Research Fellow, Ivan Kanchev DVM / Research Fellow, Jana Kopkanová MSc / Technician, Sandra Potyšová MSc / Technician, Irena Jeníčková PhD / Postdoctoral Fellow, Věra Mihálová / Technician, Slavomír Kinský PhD / Postdoctoral Fellow, Dominika Fričová PhD / Postdoctoral Fellow, Gizela Koubková PhD / Research Assistant
Not in the picture: Martin Balaštík PhD / Group Leader, Matej Ďurik PhD / Postdoctoral Fellow, Trevor A. Epp PhD / Group Leader, Pavlína Hermannová Diploma student, Radmila Hanečková BSc. Diploma student, Kallayanne Chawengsaksophak PhD / Group Leader, Kateřina Jeřábková MSc / PhD Student, Jana Ježková MSc / Technician, Markéta Jiroušková, Iris M. Manosalva-Peña PhD / Postdoctoral Fellow, Irena Placerová MSc / Technician, Jan Polák PhD / Research Associate, Pavla Rachačová MSc / Technician, Marie Řadová MSc / PhD Student, Maja Sabol PhD / Postdoctoral Fellow, Erik Šebrle Diploma student, Ondřej Šeda PhD / Group Leader, Kateřina Škarabellová Diploma student, Romana Weissová MSc / Technician

Laboratory of RNA Biology

Pre-mRNA splicing, spliceosome, epigenetics, nuclear architecture, retinitis pigmentosa

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Our long-term interest is to determine how cells decode information stored in the genome. We focus on the molecules called mRNAs that serve as messengers between DNA and proteins. Information for protein synthesis in our genome is fragmented and the coding sequences are joined together after transcription of DNA into RNA in a process called RNA splicing. In our laboratory, we analyse how the protein coding fragments are recognized and joined together. We mainly focus on how the nuclear environment and mainly chromatin influence RNA splicing, and the quality control mechanisms that ensure that the splicing machinery is correctly formed on proper RNA. These studies also help us to understand why mutations in proteins that catalyse RNA splicing cause retinitis pigmentosa, a human genetic disease characterized by photoreceptor cell degeneration. As we mostly study all these processes directly in living cells, we widely employ cell culture and various microscopy techniques (e.g. super-resolution fluorescence microscopy, live cell imaging, high-content microscopy, and other).

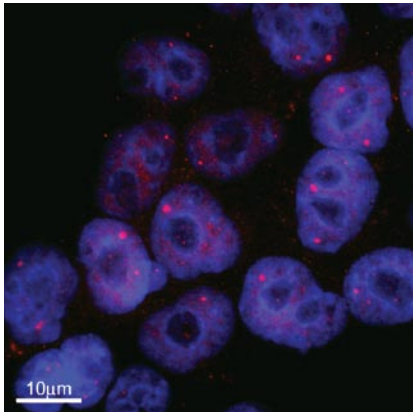

Fig. 1. Distribution of the splicing factor LSm4 (red) in cancer cells. The cell nucleus is visualized by DNA staining (blue), Cajal bodies are the bright red spots. Classical fluorescence microscopy.

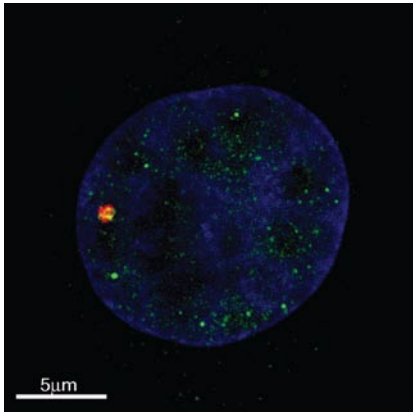

Fig. 2. Localization of the splicing factor U2 snRNA (green) in the cell nucleus (blue – DNA) and in Cajal bodies (red) using structured illumination-based super-resolution microscopy. One cancer cell shown only.

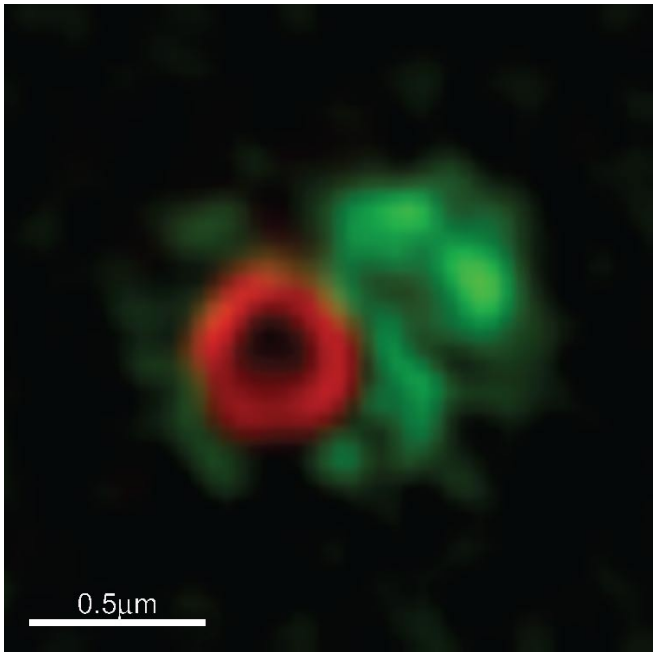

Fig. 3. Two spliceosome assembly proteins (SART3 and SMN) visualized in the Cajal body by structured illumination-based super-resolution microscopy.

- GACR, GAP302/11/1910 – Formation of splicing machinery in the context of the cell nucleus, 2011-2014, D. Staněk
- GACR, GBP305/12/G034 – Centre for RNA Biology, 2012-2018, D. Staněk, P. Svoboda
- GACR, GPP301/12/P425 – Functional analysis of hBrr2 mutations linked to retinitis pigmentosa, 2012-2014, Z. Cvačková
- ASCR, M200521206 – Functional organization of nuclear Cajal bodies with focus on the formation of ribonucleoprotein particles, 2012-2014, D. Staněk
- MEYS, LH14033 – Spliceosomal snRNP assembly and surveillance in Drosophila melanogaster, 2014-2016, D. Staněk
- GACR, GAP305/10/0424 – Regulation of alternative splicing via chromatin acetylation, 2010-2013, D. Staněk
- GAUK, 652213 – Aire, alternative splicing in the immune system, 2013-2014, S. Hozeifi/K. Klimešová
- GAUK, 460413 – SMN role during maturation of snRNPs in the cell nucleus, 2013-2015, A. Malinová
- GAUK, 320713 – Early stages of spliceosome formation, 2013-2014, E. Stejskalová

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From the left: Anna Malinová, MSc / PhD Student, Eva Kozáková (Dušková), PhD / PhD Student (until 2014)/ Postdoctoral Fellow (since October 2014), Nicole Bieberstein, PhD / Postdoctoral Fellow (since 2013), Martin Volek / Diploma Student (since 2014), Zuzana Cvačková, PhD / Research Associate, Klára Klimešová / Diploma Student, Zuzana Šándorová MSc / PhD Student (since 2014), Adriana Roithová MSc / Diploma Student (until 2014)/ PhD Student (since 2014), Eva Stejskalová (Šimková), MSc / PhD Student.

Not in the picture: David Staněk, PhD / Head of Laboratory, Samira Hozeifi, MSc / PhD Student (until 2014), Ivan Novotný, PhD / Postdoctoral Fellow (until 2013), Daniel Matějů / Diploma Student (until 2013)

Laboratory of Epigenetic Regulations

RNA degradation, dsRNA, RNAi, miRNA, chromatin

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We study mechanisms governing the gene expression control during mammalian oocyte-to-embryo transition (OET, Fig. 1). Mammalian OET is an orchestrated process where a highly specialized cell – the oocyte – is transformed into a cell that will develop into a new organism. Importantly, oocyte reprogramming is closely associated with producing the pluripotent nature of embryonic cells, i.e. their ability to differentiate into any body cell type. We currently work on three OET topics:

Maternal mRNA metabolism

OET relies on extensive post-transcriptional control of maternal mRNAs. Maternal mRNAs, which are no longer needed, are eliminated while mRNAs, whose products are needed for zygotic genome activation (ZGA), are maintained and translated (Fig. 2). We focus on the molecular foundations of selective mRNA degradation. While some maternal mRNAs are naturally unstable, others are relatively stable and their degradation occurs in waves triggered by three major developmental transitions: resumption of meiosis, fertilization, and ZGA. We aim to develop an integrated model based on the dynamics of mRNA degradation pathways, mRNA binding proteins, and combinatorial composition of 3'UTR motifs, which would explain the observed maternal mRNA behaviour.

Role of small RNAs during OET

We study the role of small RNAs (microRNAs (miRNAs), short interfering RNAs (siRNAs) and PIWI-associated RNAs (piRNAs)) in the mammalian female germline. Mouse oocytes offer a unique co-existence of all three classes of small RNAs. It was reported that miRNA activity is minimal and non-essential in mouse oocytes, while endogenous siRNAs are required for normal meiotic maturation. We discovered the cause of highly active RNAi in mouse oocytes – a unique truncated maternal isoform of Dicer, the key enzyme in siRNA biogenesis (Fig. 3). Such an isoform was not found in other mammals except rats. Our current research addresses endogenous RNAi-related questions: How do the small RNAs function in oocytes of other mammals? Which consequences would bring enhanced RNAi in somatic cells? Can we use chemical biology to modulate RNAi and miRNA pathways?

Role of long non-coding RNAs during OET

Long non-coding RNAs (lncRNAs) are a heterogeneous group of genome-encoded RNAs, many of which have important biological functions. In collaboration with the laboratory of Kristian Vlahovicek from the Zagreb University (bioinfo.hr), we explored microarray and next-generation sequencing datasets and generated a catalogue of lncRNAs expressed during OET. Remarkably, many of the identified lncRNAs are novel and have unique expression patterns. We currently work on functional analysis of selected candidates.

This research is supported by a Marie Curie Initial Training network, RNATRAIN.

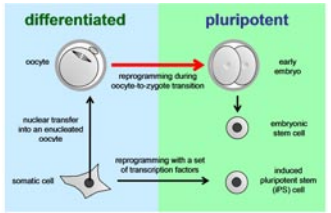
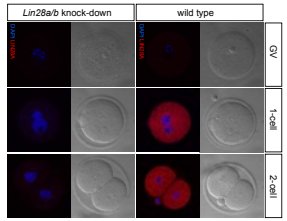


Fig. 1. Oocyte-to-zygote transition is a unique model for studying pluripotency. The mammalian oocyte is a highly specialized cell, whose cytoplasm is capable of reprogramming a genome to initiate development of a new organism. The blastomeres of the 2-cell embryo are totipotent as they can give rise to all 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. Lin28a dormancy. Lin28 mRNA stored in the oocyte is recruited for translation after fertilization



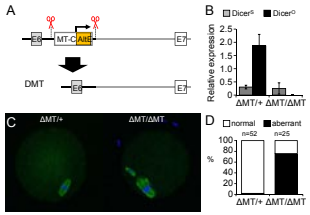


Fig. 3. Specific retrotransposon element deletion phenocopies conditional Dicer deletion in mouse oocytes. (A) Schematic depiction of cleavage sites for specific TAL effector nucleases (depicted as scissors) to generate the •MT allele. (B) Truncated Dicer (DicerO) isoform expression is absent in •MT/•MT oocytes. (C, D) Spindle defects frequently appear upon loss of DicerO.

- GACR, GBP305/12/G034 – Centre for RNA Biology, 2012-2018, D. Staněk, P. Svoboda
- ASCR, M200521202 – Integrative approach to understanding the mechanism of genome activation and natural occurrence of pluripotency in mammalian embryo, 2012-2015, P. Svoboda
- FP7 EU, 607720 RNATRAIN – The European non-coding RNA network, 2013-2017, P. Svoboda
- GACR, GA13-29531S – Development of chemical regulators of miRNA and RNAi pathways, 2013-2016, P. Svoboda
- MEYS, LH13084 LH - KONTAKT II – Post-transcriptional control of oocyte-to-zygote transition, 2013-2015, P. Svoboda
- GACR, GA204/09/0085 – RNA silencing and long dsRNA in mammalian cells, 2009-2013, P. Svoboda
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From the left: Petr Svoboda, PhD / Head of Laboratory, Kateřina Chalupníková, PhD / Postdoc (maternity leave), Ondřej V. Šolc, Sravya Ganesh / PhD Student (since 2014), Radek Malík, MD, PhD / Postdoc, Eliška Svobodová, MSc / PhD Student (since 2013), Josefina A. "Shotgun" Šolcová, Radek Jankele / Diploma Student, Jana Faltýnková / Diploma Student, Kateřina Podolská, MSc / PhD Student (maternity leave), Jana Nejepínská, MSc / Postdoc, Michaela Vaškovičová/ Diploma Student

Not in the picture: Jan Petržílek, Meyer Lansky / Secretary, Claire Louise Ryan, MSc / PhD Student (until 2014)

Laboratory of Genomics and Bioinformatics

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

Čestmír Vlček

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To understand the evolution of eukaryotes and the developmental processes that they regulate, it is necessary to analyse their genomes and transcriptomes. Genome sequences are the ultimate source for phylogenomics. 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. Representative genome sequences are still limited or altogether lacking for a large number of lineages. 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 molecular diagnostics and personalized medicine. We study intracellular interactions in malignant melanoma and in tumour-associated fibroblasts using genomics tools.

Fig. 1. A protozoan genome project: Mastigamoeba eukaryotic cell photo.

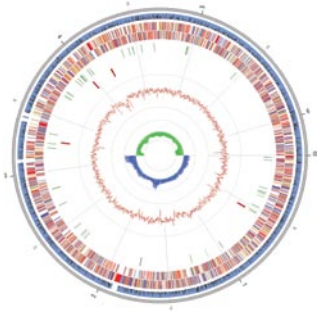

Fig. 2. The genome of a phenol derivative-degrading bacterium, Rhodococcus erythropolis strain CCM2595. This bacterium is interesting in the context of bioremediation for its capability to degrade phenol, catechol, resorcinol, hydroxybenzoate, hydroquinone, p-chlorophenol, p-nitrophenol, pyrimidines, and sterols.

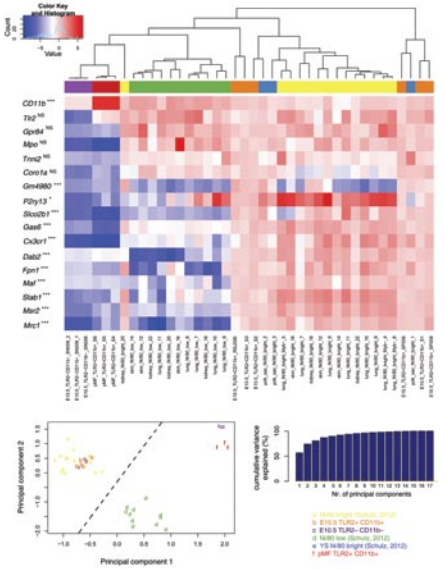

Fig. 3. Microarray analysis of E10.5 TLR2+ CD11b+ macrophages. A comparative analysis of gene expression profile of different types of macrophages using two independent microarray datasets.

- GACR, GAP506/11/1317 – Diversity and evolution of anaerobic Heterolobosea, 2011-2014, Č. Vlček
- GACR, 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
- GACR, GAP506/11/1320 – Establishment of the secondary plastid in euglenids, 2011-2015, Č. Vlček
- GACR, GAP304/12/1333 – Intercellular interactions in malignant melanoma – experimental study, 2012-2015, H. Strnad
- GACR, GAP506/12/1010 – Genome sequencing of oxymonad and Trimastix, 2012-2014, V. Pačes
- MH, NT13488 – Genomic analysis of tumour-associated fibroblasts in head and neck carcinoma: the basis for new generation of biologic anti-tumour therapy, 2012-2015, H. Strnad
- MH, NT13112 – Studies of anticancer effects of statins, 2012-2015, H. Strnad
- GACR, GA13-20293S – Cellular and molecular characteristics of neonatal human skin: consequences for skin healing, 2013-2016, H. Strnad
- GACR, GA13-33039S - A genomic approach to unravelling the biology and evolution of eustigmatophyte algae, 2013-2015, V. Pačes
- GACR, GA13-24983S – Unravelling the early evolution of the eukaryotic cell through exploring the genomes of the eukaryotic superphylum Discoba, 2013-2016, Č. Vlček
- GACR, GA13-28283S – Bridging microbial community ecology and degradation of xenobiotics – the use of metagenomics to investigate microbial degradation potential, 2013-2017, M. Kolář
- MEYS, LG14017 LG-INGO II – Ensuring representation of the Czech scientific community in FEBS, IUBMB, EMBC, EMBO, ESBRA, and relevant organizations, 2014-2016, V. Pačes
- TACR, TE02000058 – Centre of competence for molecular diagnostics and personalized medicine, 2014-2019, V. Pačes, Č. Vlček

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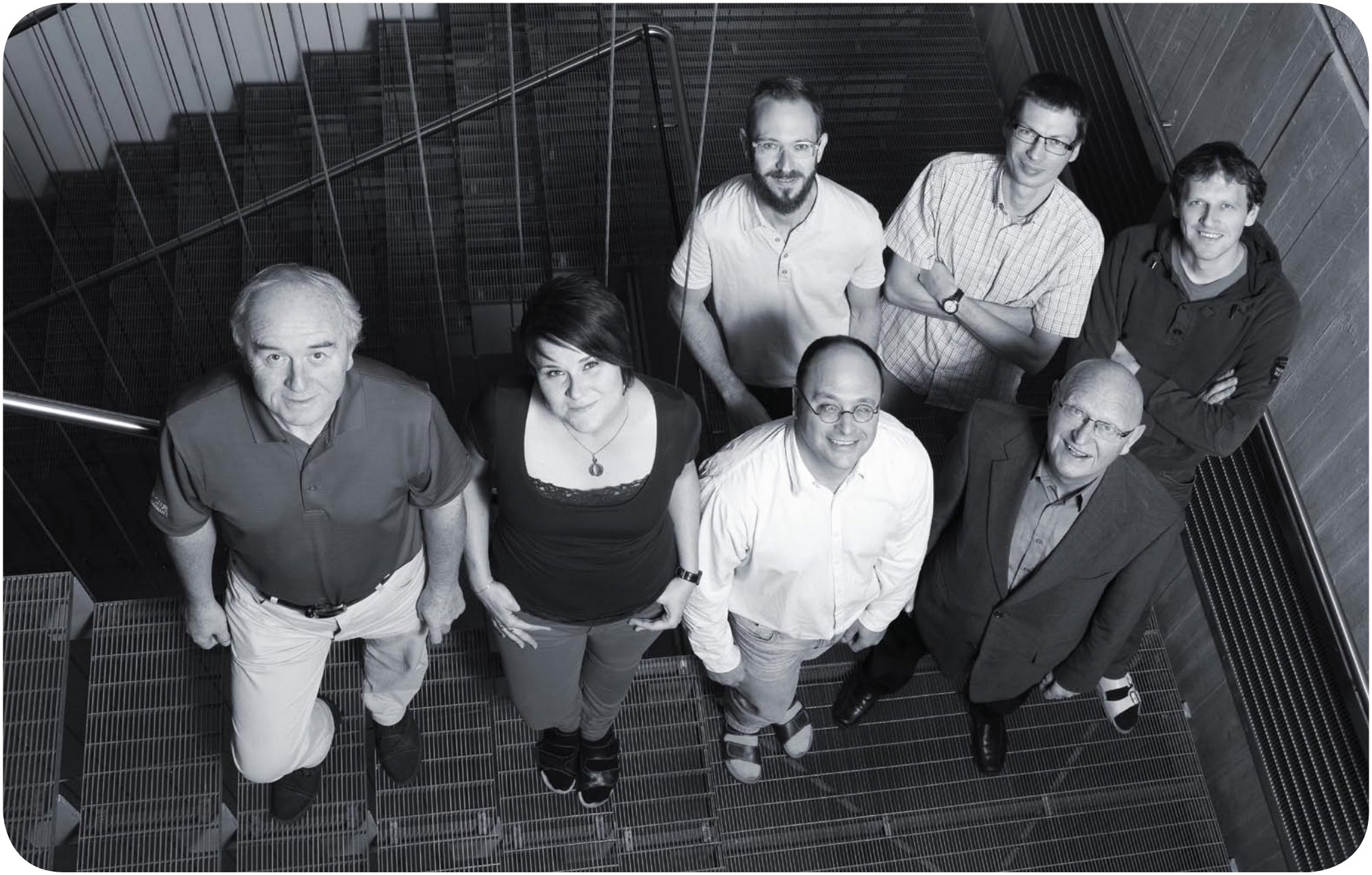
2. Hron T, Fábryová H, Pačes J, Elleder D: Endogenous lentivirus in Malayan colugo (Galeopterus variegatus), a close relative of primates. **Retrovirology 2014** 11(1): 84.
3. Strnad H, Patek M, Fousek J, Szokol J, Ulbrich P, Nesvera J, Pačes V, Vlček Č: Genome Sequence of Rhodococcus erythropolis Strain CCM2595, a phenol derivative-degrading bacterium. **Genome Announc 2014** 2(2).
4. Nývltová E, Šuták R, Harant K, Šedinová M, Hrdy I, Pačes J, Vlček Č, Tachezy J: NIF-type iron-sulfur cluster assembly system is duplicated and distributed in the mitochondria and cytosol of Mastigamoeba balamuthi. **Proc Natl Acad Sci USA 2013** 110(18): 7371-6.
5. Zubáčová Z, Novák L, Bublíková J, Vacek V, Fousek J, Rídl J, Tachezy J, Doležal P, Vlček Č, Hampl V: The mitochondrion-like organelle of Trimastix pyriformis contains the complete glycine cleavage system. **PLoS One 2013** 8(3): e55417.



From the left: Čestmír Vlček, PhD / Head of Laboratory, Miluše Hroudová, PhD / Postdoctoral Fellow, Jakub Rídl, MSc / PhD Student, Jan Pačes, PhD / Research Fellow, Michal Kolář, PhD / Research Fellow, Prof Václav Pačes, DSc / Research Fellow, Hynek Strnad, PhD / Research Fellow

Not in the picture: Šárka Pinkasová / Technician, Jana Šáchová, MSc / PhD Student, Mirka Famfulíková / Diploma Student, Martin Šteffl, MD / PhD Student

Information Technologies

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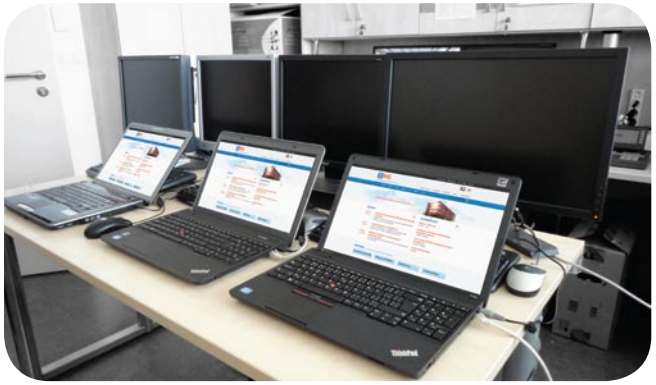
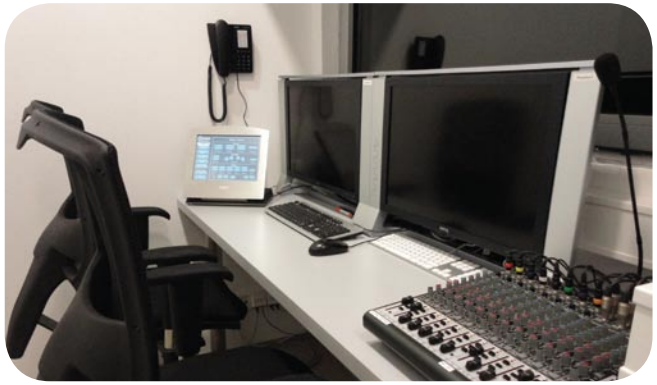
The IT department provides a wide range of information technology services to support various needs of the users in the Institute. The main tasks include the administration of LAN and wireless network in the img.cas.cz domain, administration of institutional servers and storage area network (SAN) infrastructure, as well as performing data backup and archiving. The critical hardware equipment is housed in two modern data centre rooms with controlled air-conditioning, uninterrupted power supply, temperature and humidity monitoring, and fire protection system. On a daily basis, the IT department ensures the installation and registration of computers and printers to the computer network, hardware purchase consultancy, and technical support for users of PC and Mac platforms. The volume or site licenses for commonly used software in the Institute are arranged. Particular support is also provided to the other service units and research groups that includes development of websites and web applications, support for internal databases (e.g. animal tracking system), operation of access control system and surveillance system. In addition, the IT department operates the audio-visual equipment in the conference hall and provides IT assistance and hardware equipment for courses and conferences organized at the Institute.

Main data centre room

Disk storage system



Installation of laptops



Control room of the conference hall

From the left: Tomáš Volf (since 2014), Jan Šveňha (since 2013), Jiří Růžička (since 2014), Michal Kůs, Michal Žáček (since 2013), Michal Rolník, Juraj Kopčan, MSc, Petr Janků, MSc, Jan Hurda (since 2013), Petr Divina, PhD / Head

Not in the picture: Miroslav Indra, PhD

Genomics and Bioinformatics

Hynek Strnad

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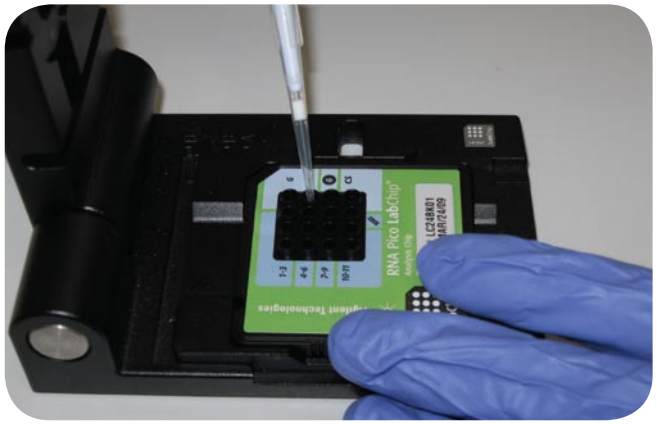
The facility was established in late 2005 after purchase of the Affymetrix GeneChip System and was initially operated by the staff from the Department of Mouse Molecular Genetics. Since January 2007, it has become an independent unit which provides full chip microarray services, real-time quantitative 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 cyclers Roche LC480, JANUS robots, EnVision Plate Reader from PerkinElmer and QX200 Droplet Digital PCR System, and also with instruments for assessment of quality and quantity of the processed samples (spectrophotometer Nanodrop, Qubit Fluorometer and capillary electrophoresis Agilent Bioanalyzer 2100).

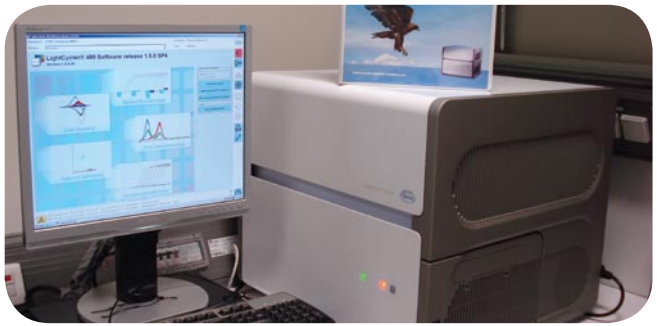
Illumina BeadArray Reader

Affymetrix GeneChip Scanner



Agilent Bioanalyzer

QX200 Droplet Digital PCR System



LightCycler LC480 II for real-time PCR

From the left: Šárka Kocourková, MSc, Hynek Strnad, PhD / Head, Marcela Vedralová, MSc

Not in the picture: Martina Chmelíková, MSc

Monoclonal Antibodies and Cryobank

Dobromila Kumpoštová

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www.img.cas.cz
/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, O_2 concentration) and safety both in the cryobank and in the individual storage containers are followed by the monitoring system with GSM and web interface outputs.

Cryobank equipment

Cell culture supernatant

Cryobank equipment

From the left: Ladislava Sobotková, Dobromila Kumpoštová, MSc / Head, Hana Korábová

Histological Laboratory

Vladimír Kořínek

vladimir.korinek@img.cas.cz

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

The laboratory is equipped for tissue dehydration, preparation 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.

Paraffin embedding station

Rotary microtome with equipment

Tissue processor for dehydration

Media and Glass Washing

Hana Marxová

hana.marxova@img.cas.cz

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: Lenka Alferiová, Hana Marxová, Jitka Škopová, Stanislava Bendová

Animal Facility

Miloslava Vilhelmová

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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. The facility produces eggs, embrya and chickens needed by several research groups focusing on chicken models.

Breeding of adult animals

White Leghorn hen

Brown Leghorn cock

From the left: Iva Hrodková, Renata Matzeková (since 2013), Jitka Dvořáková, Radmíra Skoková, Alena Eisensteinová, Miloslava Vilhelmová, MSc, CSc / Head, Eva Bernášková

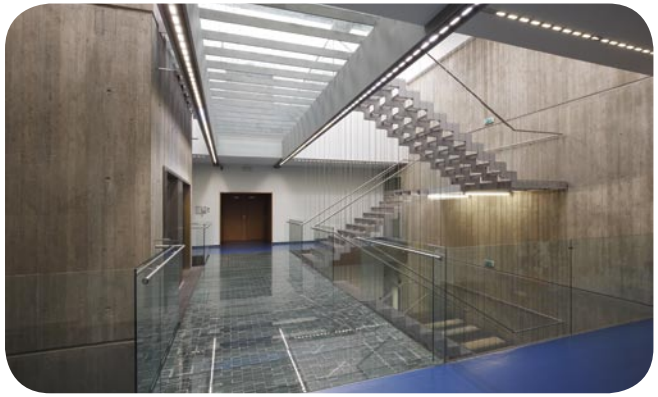
Not in the picture: Martina Mináriková, MSc / Head (maternity leave), Kamila Thunová, Jaroslava Vlasáková, Petra Faloutová (until 2013), Zdena Koptová (until 2013), Alena Porazilová (until 2013), Jaroslava Strnadelová (until 2013),

Building Maintenance

Jana Boučková

jana.bouckova@img.cas.cz

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



From the left: Marie Kasková, Jitka Sedláčková, Jana Boučková, Jiřina Kozyková

Not in the picture: Miroslav Heyduk, Jiří Macek, MSc, Dana Macková, Daniela Macková

Office of the Director

Ilona Dita

ilona.dita@img.cas.cz

www.img.cas.cz/core-facilities/office-of-the-director

From the top left: Leona Krausová, Gabriela Marešová, Šárka Takáčová, MSc, Prof Jiří Jonák, MD, DSc, Vendulka Svobodová, MSc, Jana Jeglová, MSc (since 2013), Kateřina Sedláčková, Ilona Dita (since 2014), MSc, Šárka Šímová, PhD

Not in the picture: Jitka Černá (until 2014)

Finances and Administration

Jana Immerová

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www.img.cas.cz/core-facilities/economy-department

From the top left: Martina Málková (since 2014), Michal Švestka, Emílie Štorchová, Jitka Třísková, Michal Sedláček (since 2014), Hana Nezbedová, Iva Palacká (until 2014), Klára Knížková, Jana Immerová / Head, Lucie Hoferiková (since 2014), Veronika Takáčová, Markéta Ondráčková, MSc (since 2014), Milena Petríková, Lenka Zahurská (since 2014)

Not in the picture: Ivana Brabencová, Pavlína Ježková (since 2014), Kateřina Drastilová (until 2014), Hana Švestková (maternity leave)

BIOCEV Division

Pavel Martásek

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

Cancer, cardiovascular diseases, viruses, infections – those are some of the most pressing problems of human population. In the past, we were only able to describe these problems, not find their cause. Today, we are able to understand the origins of the diseases down to the molecular level. This allows us to design solutions that may ultimately lead to producing the right drug or determining the best treatment method and, by extension, saving many lives.
Prof Pavel Martásek, MD, DSc, Director of BIOCEV

A Hot Spot of Science in the Heart of Europe
In September 2009, IMG established the BIOCEV Division. Its main task is to ensure successful implementation of a new European scientific centre of excellence in the field of biotechnology and biomedicine, whose outputs will lead to better quality of life and to development and growth of both the knowledge economy and competitiveness of the Czech Republic. Its implementation is a collaborative effort of six institutes of the Academy of Sciences of the Czech Republic and two faculties of Charles University in Prague. The uniqueness of the project lies in its balanced combination of research, education and intensive cooperation with the commercial sector. Led by renowned experts, BIOCEV research teams have access to cutting-edge technologies and are an active part of major European groupings.
BIOCEV will become a platform that brings together scientists, students and corporate representatives. Interaction between these groups is crucial for successful discoveries.

Pillars of the Project BIOCEV

TEACHING AND EDUCATION
- A wide range of educational activities and development of new graduate and postgraduate programmes of study
- Training for private sector employees
- Popularization and media coverage of biotechnology and biomedical fields

RESEARCH AND DEVELOPMENT
- Five biotechnology and biomedical research programmes: functional genomics, cell biology and virology, structural biology and protein engineering, biomaterials and tissue engineering, and development of treatment and diagnostic procedures
- Fully-equipped core facilities with cutting-edge instruments: Czech Centre for Phenogenomics, Centre of Molecular Structure, Centre of Imaging Methods, OMICS Laboratory, Cryotechnologies and Quantitative and digital PCR core facility
- Integration into the European Research Area: e.g. Infrafrontier, Instruct and the Euro-BioImaging consortium

TECHNOLOGY TRANSFER
- Transfer of the results of basic research into practice (human and veterinary medicine)
- Intensive cooperation between companies and BIOCEV research facilities
- Protection of intellectual property

BIOCEV is concentrating on strategic collaboration with Czech and foreign partners in the following fields:
- Staff exchange research fellowships
- Mutual exchange of students of doctoral courses of study, including the opportunity to complete doctoral studies at foreign workplaces
- Postdoctoral working fellowships
- Dealing with joint research projects
- Joint output from implemented projects (in particular publications and patents)
- Sharing of technologies and development of new technologies
- Collaborative and contractual research

For more information, please see www.biocev.eu.

BIOCEV

Drugs targeted at the exact location where metabolism is damaged, joint replacements from new materials that are compatible with the body, artificial heart valves and vascular scaffolds with specially treated surfaces – those, too, will be the end results of the research being conducted at the BIOCEV centre.

Second row from the left: Tomáš Novotný, Michal Vršovský, MSc, Zbyněk Šmída, PhD, Vedran Bostandžić MA, Martin Kuthan MSc, Petr Solil MA, Miroslav Louma MSc, Martin Polák MSc, Tomáš Němec MSc, Libor Fabián MSc, Tomáš Pěnek MSc, Jan Jirků MBA, Prof. Pavel Martásek, MD, DSc, Bohuslav Vaněk MSc

First row from the left: Věra Prosová MSc, Eva Kulhavá, Markéta Nováková JD, Lenka Vosátková, Kamila Dařinová MSc, Eva Andresová, Jaroslava Malá M.A

Czech Centre for Phenogenomics

The largest biomedical infrastructure in the Czech Republic

Radislav Sedláček

radislav.sedlacek@img.cas.cz

Czech Centre for Phenogenomics

The Czech Centre for Phenogenomics (CCP) provides expertise and services to the biological and medical research community for studying the function of genes in biological processes and/or human disorders *in vivo* using mouse or rat (in preparation) models.

The basic understanding of gene functions is required for placing the recent explosion of genetic data facilitated by rapid advances in genome sequencing and linkage mapping techniques in biological context. In particular, population-based genetic associations using genome-wide association studies (GWAS) and complete sequencing of individual patient genomes can only achieve their promise for effective preventative and personalized medical intervention if the identified genes are ascribed some biological function. This represents a huge knowledge gap in the preclinical development supply chain, with at least half of mammalian genes ascribed functions that are poorly understood and nearly one-third completely unknown.

The knowledge of function of all individual genes is crucial for the future of molecular medicine and could revolutionize the development of new diagnostics and therapy. Although non-mammalian models have provided valuable knowledge on basic gene functions, the mouse, which is genetically close to humans, is ideal to model physiologic functions and diseases.

In the current era of personalized medicine, there is an increasing demand for better understanding the gene function.

CCP, which provides the Czech biomedical research community with a full spectrum of genetic engineering services, also offers open access services to a substantial number of international users.

CCP further builds its capacities and its service portfolio, and it will be one of the largest European research infrastructures for supporting genetically modified rodent (mouse and rat) research in 2015-2016. It is hosted by the Institute of Molecular Genetics and embedded in the BIOCEV project (Biotechnology and Biomedicine Centre of the Academy of Sciences and Charles University in Vestec).
Through its memberships in pan-European ESFRI (the European Strategy Forum on Research Infrastructures) infrastructure INFRAFRONTIER and the International Mouse Phenotyping Consortium (IMPC), CCP became a partner in a collective global network that aims to comprehensively and systematically analyse the effect of loss-of-function gene mutations. The goal is to produce a comprehensive 'encyclopaedia' of gene functions that will help identify causative factors of human diseases as well as novel targets for therapeutic intervention.

The service portfolio of CCP is divided into three modules:

1/ Transgenic and Archiving Module **(TAM; see page74)**
Services under TAM include large-scale (for international consortia) and targeted mutagenesis and transgenesis service on demand. Our proven record of accomplishment in custom-targeted mutagenesis includes cost-effective and fast new technologies of programmable nucleases (TALENs and CRISPR/Cas) for model generation.

2/ Phenotyping Module
The CCP is building its phenotyping capacities to accommodate both mouse and rat models.

3/ Animal Facility Module **(AFM; see page 76)**
AFM service mainly provides breeding and housing, health monitoring, biopsies, import/export service, GMO and administration.

Although the portfolio of services could be designated as "multi-purpose platform" combining approaches from various research areas, all the expertise is focused on animal models.

From the left: Radislav Sedláček PhD / Head of Laboratory, Karel Chalupský PhD / Research Fellow, Libor Daněk M.A. / Project Manager, Agnieszka Kubik-Zahorodna, PhD / Research Associate, Milan Reiniš (PhD / Group Leader), Ivan Kanchev PhD / Research Fellow, Benoit Piavaux PhD / Postdoctoral Fellow, Kallayanne Chawengsaksophak PhD / Group Leader, Trevor A. Epp PhD / Group Leader

Not in the picture: Matej Ďurík , PhD / Postdoctoral Fellow, Jan Polák PhD / Research Associate

Transgenic Archiving Module of CCP (Transgenic Unit)

Radislav Sedláček

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www.img.cas.cz/core-facilities/radislav-sedlacek

Czech Centre for Phenogenomics

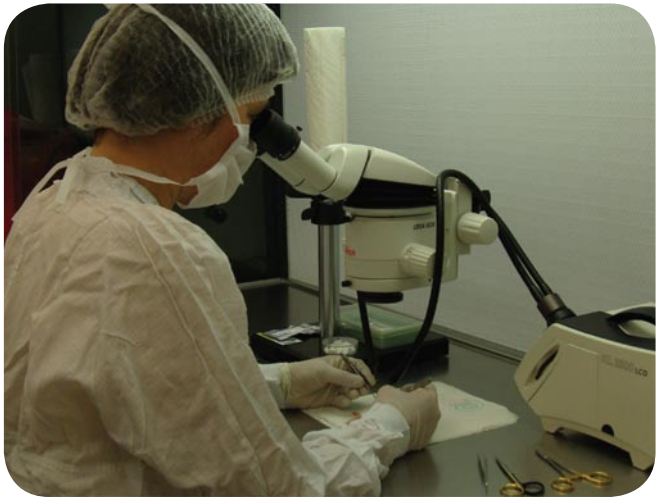
One of the key challenges in biomedical research is to attribute biological functions to the identified human genes. Although non-mammalian models have delivered valuable knowledge on basic gene functions, mouse models have become the central tool in the functional analysis of our genes and the mouse genome was fully sequenced just after the human one with the outcome that 99 % of the coding genes present in man are also present in the mouse.

CCP creates animal models with specific modifications in the genes of interest in order to decipher the role of those genes *in vivo*, or, in a more directly medically oriented approach, CCP generates models of human diseases, quite often by replicating the known mutations found in patients.

Mouse mutants with phenotypes that mimic the situation in humans have served as fundamental research tools in understanding the genetics underlying mammalian biology. These mutants represent a key in determining gene function and pathway interactions, and will continue to play a critical role, particularly by utilizing gene knock-outs. Studies utilizing single gene knock-outs or other single mutants represent fundamental steps in exploring biology of the disease system.

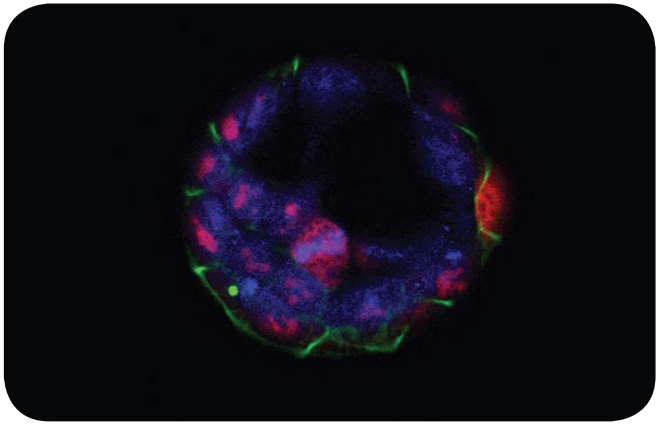
Moreover, mouse/rat models bearing single gene mutations represent a unique tool to assess and design therapeutic interventions also in diseases that involve multiple genes, and hence their protein products and targets for therapeutics. It is essential to understand which individual targets in the process may provide the maximum clinical benefit. It is well recognized that the determination of the effects of mutations in individual genes is a powerful tool for dissecting the genetic basis of the disease pathways.

The Transgenic Unit at the Institute of Molecular Genetics (TgU; **http://tgunit.img.cas.cz/**) was currently transformed into the Transgenic and Archiving Module (TAM) of the Czech Centre of Phenogenomics (CCP; **http://www.phenogenomics.cz/**) and provides a comprehensive technology portfolio. Moreover, we have invested lots of effort into the development of new technologies, specifically the technology of "programmable nucleases" such as "TALEN and CRISPR/Cas9" assisted gene targeting and genome editing, which substantially improved our services in custom-tailored targeting projects, saving cost and time. Technologies provided by TAM include embryonic stem (ES) cell derivation and manipulation, development of targeting strategies and tools, generation of transgenic animals including conditional knock-out and knock-in models, genotyping, cryo-archiving, export/import/distribution, consultancy support, and administrative support (GMO).



Surgery in production barrier (SPF facility): implantation of embryos into mouse foster mothers

Litter with chimeras



Mouse embryo, blastocyst

From the left: Henrieta Pálešová MSc / Technician, Jana Kopkanová MSc / Technician, Dana Kopperová MSc / Technician, Veronika Libová MSc / Technician, Sandra Potyšová MSc / Technician, Monika Volčková MSc / Technician, Inken M. Beck PhD / TgU Supervisor , Anna Laštůvková MSc / Technician, Radislav Sedláček PhD / TgU Head, Irena Jeníčková PhD / Postdoctoral Fellow, Björn Schuster PhD / Research Associate

Not in the picture: Irena Placerov MSc / Technician, Jana Ježková MSc / Technician

Animal Facility (Animal Facility Module of CCP)

Jan Honetschläger

jan.honetschlager@img.cas.cz

Czech Centre for Phenogenomics

The animal facility of IMG is a part of the Animal Facility Module (AFM) of the Czech Centre for Phenogenomics (CCP) and is based on the newest understanding of housing and breeding of mice and rats. The animal facility is the largest facility for rodents in the Czech Republic and provides superior standard for animal housing and breeding. Thanks to the barrier system of breeding and close cooperation with the Transgenic and Archiving Module (TAM) we have been able to house the animals with SPF health status in accordance to FELASA recommendations for more than seven years. The transgenic production site is incorporated in the main building under an SPF (specific pathogen-free) production barrier. The capacity of more than 10,000 cages allows us to breed and produce various types of transgenic mouse models including knock-out mice and including a small colony of wild-type strains for internal use.

The Animal Facility currently uses all animal facilities of IMG on the campus in Krc, Prague 4, and new capacity will be provided by the CCP building, which is being constructed within the BIOCEV project.

At the BIOCEV site, one of the most progressive animal facilities (new building to be opened in 2015) with regard to logistics, versatility, and demand for animal health will be established. Technologies employed include housing/breeding animals in individually ventilated cages (IVC), barrier system and one-way flow of material and animals, a health monitoring system, and advanced software-assisted management of animal facility operations.

After construction of the new building, CCP expects a 3-4-fold increase of capacity to provide its services and to organize specialized courses, education and PR activities.

- Animal facility service
- Breeding and housing
- Biopsies collection for genotyping and investigation
- Identification using tagging (or other standard method) of individual animals
- Import (administrative parts)
- Export (administrative parts)
- Health monitoring (using the external service)
- Administration issue regarding license/allowance for experimental work with animals

IVC housing

Mouse handling with care

From the left: MVDr. Jan Honetschläger, MBA, Daniela Vorlová, Monika Novotná, Daniela Kratochvílová, Veronika Šobíšková, Zuzana Nezbedová, Hana Černá, Jenifer Veselá, Lenka Rysslová, Bc. Alexandra Hviščová, Renáta Matoušková, Tomáš Nezbeda, Anna Koťátková, Květa Bartošová, Nikola Dušková, Ján Majerník, Zdeněk Holub. Mgr. Markéta Rynekrová. Jana Matoušková. Petr Macek. Markéta Cibulcová. Gabriela Vávrová. Dagmar Čermáková. Romana Kolaciová. MVDr. Peter Neradil. Zuzana Bakešová. Alena Babanská

Not in the picture: Jana Březinová, Stanislav Dygryn, Emilie Hájková, Pavla Kameníková, Nela Lahitová, Veronika Polívková, Věra Žbánková, Helena Žoudlíková

CZ-OPENSCREEN: National Infrastructure for Chemical Biology

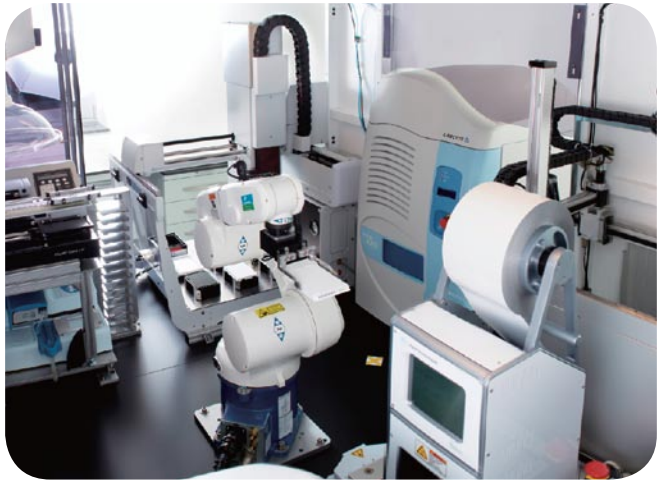
Petr Bartůněk petr.bartunek@img.cas.cz

Chemical biology is a relatively new biological discipline which has its roots in chemistry, biochemistry, cell biology and pharmacology. It basically studies the effects of chemical compounds on the living species and endeavours to provide deeper understanding of the cell behaviour, metabolic or signalling pathways, to study the underlying molecular mechanisms of cellular and organismal responses, and to identify new small molecules with specific features or develop new drugs and to treat serious human diseases.

The interdisciplinary nature of chemical biology makes the "CZ-OPENSCREEN: National Infrastructure for Chemical Biology" a "multi-purpose platform" covering all areas of biology from biochemical protein-based assays to advanced phenotype-based cellular or full organism assays, compound optimization (organic and medicinal chemistry) up to analysis (cheminformatics, bioinformatics, complex data mining).

CZ-OPENSCREEN operates the state-of-the-art technologies for basic and applied research in the fields of chemical biology and genetics and provides Open Access to the users. Since 2010, the "CZ-OPENSCREEN National Infrastructure for Chemical Biology" has been one of the priority and strategic projects included in the Roadmap for Large Research, Development and Innovation Infrastructures in the Czech Republic. Moreover, it will serve as the National node of the pan-European ESFRI infrastructure EU-OPENSCREEN, providing trans-national access to the screening platform and to the European Chemical Biology Library.

CZ-OPENSCREEN is a newly formed unit at the Institute of Molecular Genetics and is situated on the first floor of pavilion V. Its research is aimed at identifying new molecular probes/tools for research and new potential therapeutics. The portfolio of services includes access to bioactive compounds, consultancy on assay development, support in assay transfer/miniaturization, high-throughput screening (HTS) and high-content screening (HCS) campaigns with large chemical libraries consisting of more than 40,000 compounds, confirmatory dose response assays and validation experiments in vitro. Our infrastructure also provides standard profiling of newly synthetized chemical compounds and chem-/bioinformatic support and data mining (ChemGenDB/ScreenX). For more information, please visit **http://www.openscreen.cz**.



Second row from the left: Petr Bartůněk, PhD / Head, Tomáš Müller, MSc / Research assistant, Jana Bražinová Krejčová, MSc / Project Manager (since 2014), António Pombinho, PhD / Research Fellow, Petr Šálek, PhD / Project Manager (since 2014), Zuzana Kotrbová, MSc / PhD Student (since 2014), Ctibor Škuta, MSc / PhD Student, Kristýna Blažková, MSc / PhD Student (since 2014), David Sedlák, PhD / Research Fellow, Ivan Čmelo, MSc / PhD Student, Martin Popr, MSc / Research Assistent (since 2014), Olga Martínková / Technician

Not in the picture: Tomáš Bartoň, MSc / PhD Student, Jana Bartůňková, MD / Research Assistant, Dita Franke-Kidorová, MSc / Project Manager (maternity leave), Jindřich Jindřich, Assoc. Prof., PhD / Research Fellow, Michaela Marešová, MSc / Research Assistant (maternity leave), Daniel Svozil, Assoc. Prof., PhD / Research Fellow

Microscopy Centre

Pavel Hozák

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The rapid technological development in biological imaging enables observing the finest structural details, various molecules and interactions inside cells, which has not been possible so far. Indeed, biological imaging has become an essential engine that drives research in biological and medical sciences. In biology, the majority of current high-impact research publications utilizes the advanced light and electron microscopy techniques.

The Czech-BioImaging Microscopy Centre provides various modern light and electron microscopy equipment and techniques. The Centre offers a wide range of services to the local and external users from research and industrial institutions in an open access model. The research results supported by the Centre can be further applied e.g. by pharmaceutical sector in developing new antiviral vaccines, anticancer drugs, diabetes cure, etc. The Centre widely cooperates with industry for improvement of the available methods and for intensive elaboration of the new tools.

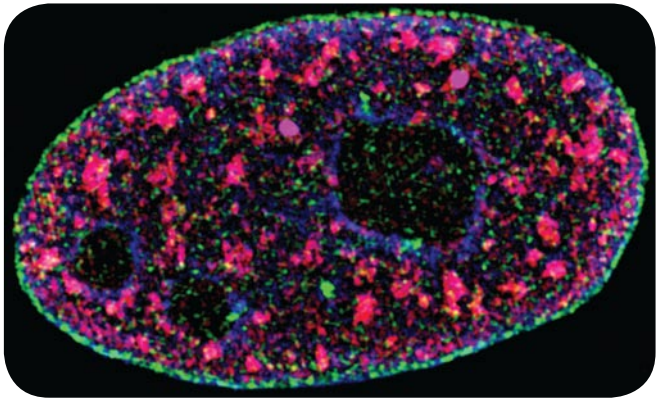
The Centre has a strong background in organizing courses and training focused on both theoretical and practical aspects of basic and advanced microscopy techniques. In January 2014, a new Nikon Centre of Excellence in Super-Resolution Microscopy was established within the framework of cooperation between the Nikon Company and the Institute of Molecular Genetics AS CR. The super-resolution microscope is extensively used for various research and industrial tasks.

The Czech-BioImaging Microscopy Centre consists of two facilities:

- **Electron Microscopy Facility**
- **Light Microscopy and Flow Cytometry Facility**

Based on the achieved results, continuing collaborations, own developed technologies and existing equipment, the Czech-BioImaging Microscopy Centre intends to extend the research topics and the geographic areas of cooperation, industrial sector included. The Centre plans to introduce new methods and techniques corresponding to the demands of the research and industrial users.

The Centre will also function as a hub of the national large research infrastructure for biological and medical imaging (Czech-BioImaging) that brings together leading imaging facilities in the Czech Republic. It is also a candidate node of the pan-European imaging infrastructure Euro-BioImaging.



From the left front row: Lenka Pišlová, Markéta Morská, Margaryta Sobol, Zuzana Lubovská

From the left second row: Ivan Novotný, Ondrej Horváth, Pavel Hozák, Vlada Filimonenko, Anatoly Filimonenko, Ivana Nováková, Zdeněk Cimburek

Microscopy Centre - Electron Microscopy

Margaryta Sobol

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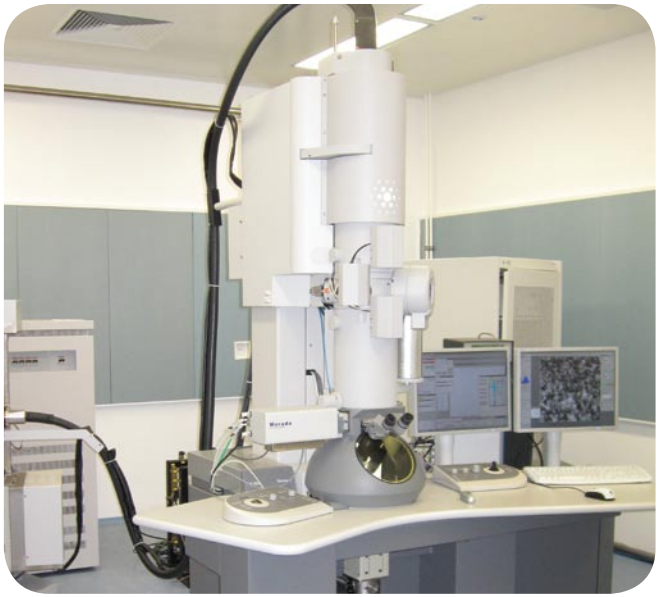
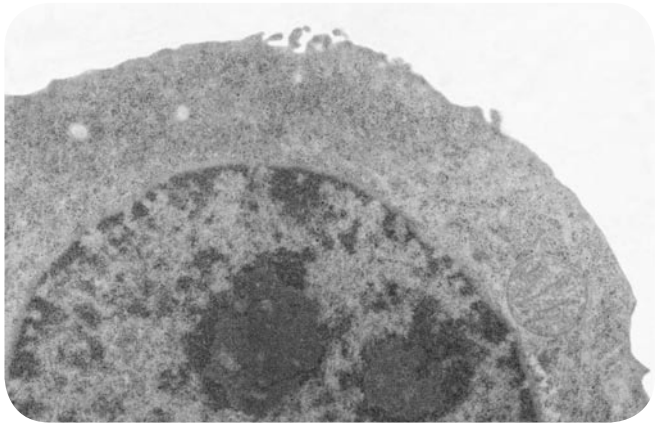
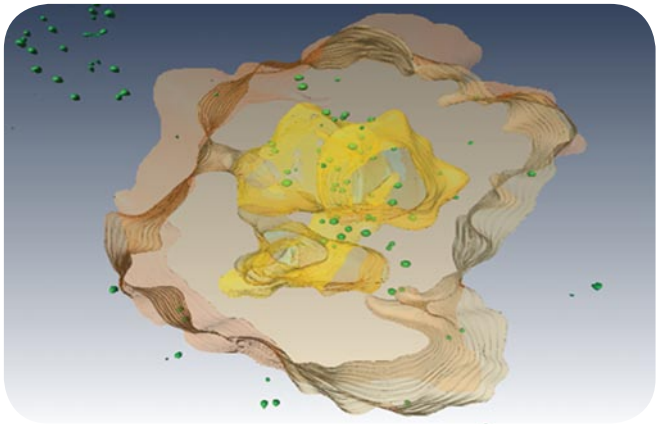
The Electron Microscopy Core Facility provides expertise and cutting-edge equipment for a broad range of biological sample preparation and ultrastructural imaging techniques. The core facility deals with various biological samples: human and animal cell cultures, plant and animal tissues, worms, microorganisms, lipid micelles. The sample preparation techniques include routine chemical fixation and resin embedding, cryo-fixation using the high-pressure freezing technique, freeze-substitution, plunge-freezing, cryo-sectioning, and immunolabelling, including simultaneous detection of multiple targets by our self-developed methods.

Our team has a long expertise in the development and optimization of sample preparation techniques for electron microscopy. We have optimized cryo-fixation of cultured cells and sample processing after cryo-fixation. Furthermore, we have developed a completely new system for simultaneous immunolabelling of five molecular targets and a special technique for combination of 3D pre-embedding immunolabelling and 2D on-section immunolabelling to provide additional possibilities for spatial analysis of biological processes. Currently, we also collaborate with the companies designing instrumentation for electron microscopy with the aim to improve sample preparation for various modes of microscopic analysis.

We collaborate with visiting scientists from the Czech Republic, Croatia, France, Japan, Switzerland and others. We cover the following topics: cellular mechanisms of infection by *Francisella tularensis*; heavy metal uptake by plant roots; mechanisms of apoptosis induction; regulation of p62 in dividing and terminally differentiated cells; mechanisms of spermatogenesis; cellular mechanisms of diabetes; analysis of antigen presence in viral vaccines; study of the structure of lipid micelles with respect to their suitability for DNA transfection; compartmentalization of actin-related proteins in the cell nucleus and their involvement in chromatin compartmentalization. The Electron Microscopy Core Facility holds the leading position in the areas concerning participation of phosphoinositides, actin, and myosin 1c in the organization and functions of the cell nucleus.
A high-pressure freezing machine, two automatic freeze-substitution machines, cryo-ultramicrotomes, Vitrobot for automated plunge-freezing, and additional wet lab equipment are available. The core facility is equipped with two transmission electron microscopes (TEM) –standard instruments for routine observation – and an advanced 200 kV instrument providing the possibility of high-resolution TEM, 3D electron tomography, cryo-electron microscopy and electron energy-loss (EELS) analysis.
We organize regular theoretical and practical courses "Microscopy Methods in Biomedicine", "Transmission Electron Microscopy in Life Sciences", "Processing and Analysis of Microscopic Images in Biomedicine", "Microscopy Immunodetection in Biomedicine" for both undergraduate and postgraduate students and for research fellows.







Microscopy Centre - Light Microscopy and Flow Cytometry

Ondrej Horváth

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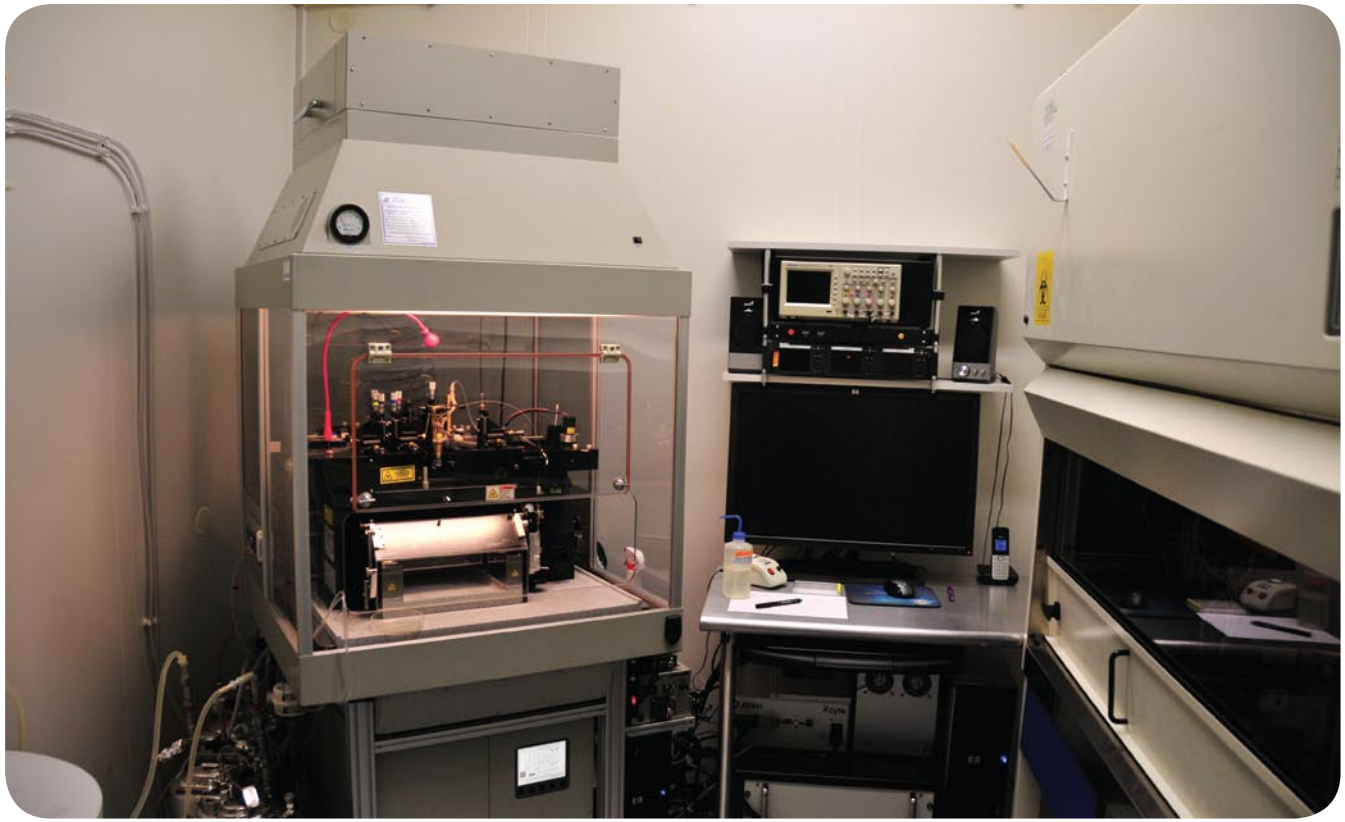
The facility provides methodological and instrumentation background for flow cytometry and fluorescence microscopy techniques. The facility is equipped with three flow cytometers – two analysers (BD FACSCalibur and BD LSRII) and one sorter. The LSRII instrument is the four-laser (405, 488, 561 and 633-nm) type with 14 fluorescence detectors. A large set of dichroic mirrors and bandpass 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 using 96- or 384-well plates. Polychromatic high-speed cell sorter BD-Influx is equipped with five lasers (355, 405, 488, 561 and 640 nm), 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 light microscopy part of the facility is currently running ten microscopy systems. Recently, we have introduced super-resolution microscopy techniques into routine use – N-SIM structured illumination microscope (a microscope provided by Nikon, Nikon Centre of Excellence run as part of the facility) and STED (stimulated emission depletion) microscope (Leica TCS SP8 X STED3X WLL). We run several confocal systems: laser scanning confocal microscope with fast scanner and three supersensitive HyD detectors (Leica TCS SP5 AOBS TANDEM), Leica TCS SP8 confocal microscope and Nikon AZ-100 zoom microscope system with confocal scanner C2 (provided by Nikon, Nikon Centre of Excellence run as part of the facility). Other microscopy systems include Leica inverted fluorescent microscope with TIRF illumination (Leica TIRF MC), wide-field inverted fluorescence microscope with laser photo manipulation (DeltaVision Core), prototype of multimodal holographic microscope (Tescan), laser micro dissection microscope (Leica LMD6000) and high-throughput microscopy system ScanR (Olympus). Several 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 super-resolution imaging SIM and STED, 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, LAS AF, Huygens, Metamorph, ImageJ).



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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:

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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.

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Publications 2013-2014

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Seminar Speakers 2013

18/01/13	**Tomasz Schneider**	(Nuffield Department of Clinical Neurosciences, Oxford University, Oxford, UK)
23/01/13	**Petr Daněček**	(Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK)
01/02/13	**Ondřej Šeda**	(First Faculty of Medicine, Charles University, Prague)
19/03/13	**Josef Lazar**	(Institute of Plant Molecular Biology, Biology Centre of the ASCR, v. v. i., České Budějovice; Institute of Nanobiology and Structural Biology of Global Change Research Centre of the ASCR, v. v. i., Nové Hrady)
28/03/13	**Heiko Lickert**	(Helmholtz Zentrum München, German Research Centre for Environmental Health, Institute of Stem Cell Research, Neuherberg, Germany)
20/05/13	**Monique Zetka**	(Department of Biology, McGill University, Montreal, Quebec, Canada)
05/06/13	**Richard Clarkson**	(Schools of Biosciences and Pharmacy, University of Cardiff, Cardiff, Wales, UK)
12/06/13	**Mark R. Opp**	(Department of Anesthesiology & Pain Medicine, University of Washington, Seattle, WA, USA)
21/06/13	**Daniele Fabris**	(RNA Institute, State University of New York, New York, NY, USA)
25/06/13	**Jens Volker**	(Rutgers Department of Chemistry and Chemical Biology, State University of New Jersey, Piscataway, NJ, USA)
11/07/13	**Michael Reth**	(University of Freiburg, Max-Planck-Institute of Immunobiology and Epigenetics, Freiburg, Germany)
28/08/13	**Paul Saftig**	(Biochemical Institute of Christian – Albrechts - Universität Kiel, Kiel, Germany)
02/10/13	**Ed Palmer**	(University Hospital Basel and University of Basel, Basel, Switzerland)
11/10/13	**Anton J. M. Roks**	(Erasmus University, Rotterdam, Netherlands)
14/10/13	**Victoria Moreno Manzano**	(Centro de Investigacion Príncipe Felipe, Valencia, Spain)
22/10/13	**Jiří Neužil**	(Institute of Biotechnology of the ASCR, v. v. i.; School of Medical Science, Griffith University, Brisbane, Australia)
30/10/13	**Ludger Klein**	(Institute for Immunology, Ludwig-Maxmilians-Universität, Munich, Germany)
04/11/13	**Ian Adams**	(MRC Human Genetics Unit, Institute of Genetics and Molecular Medicine, University of Edinburgh, Western General Hospital, Edinburgh, Scotland, UK)
12/11/13	**Arne Skerra**	(Technische Universität München, Munich, Germany)
15/11/13	**Jakub Abramson**	(Weizmann Institute of Science, Rehovot, Israel)

Seminar Speakers 2014

09/01/14	**Jean-Marie Buerstedde**	(Department of Immunobiology, Yale University School of Medicine, New Haven, CT, USA)
26/03/14	**Jozef Nosek**	(Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia)
28/03/14	**Patrick Lemaire**	(Centre de Recherche de Biochimie Macromoléculaire - UMR 5237, Montpellier, France)
02/04/14	**Marcus Groettrup**	(Department of Biology/Immunology, University of Konstanz, Konstanz, Germany)
09/04/14	**Attila Toth**	(Technische Universität Dresden, Dresden, Germany)
30/04/14	**Katarína Mikušová**	(Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia)
20/05/14	**Jan Frič**	(Singapore Immunology Network, Singapore)
21/05/14	**Edouard Bertrand**	(Institut de Génétique Moléculaire de Montpellier CNRS-UMR 5535, Montpellier, France)
04/06/14	**Marek Jindra**	(Institute of Entomology, Biology Centre of the ASCR, v. v. i., České Budějovice)
18/06/14	**Marcos Malumbres**	(Spanish National Cancer Research Centre (CNIO), Madrid, Spain)
30/07/14	**Vladimír Varga**	(Sir William Dunn School of Pathology, University of Oxford, Oxford, UK)
06/08/14	**Leonard I. Zon**	(Children's Hospital Boston, Boston, MA, USA)
27/08/14	**Jan Šilhán**	(MRC Laboratory of Molecular Biology, Cambridge, UK)
27/08/14	**Jan Frič**	(Singapore Immunology Network, Singapore)
11/09/14	**Natalia A. Bulgakova**	(Wellcome Trust/Cancer Research UK, Gurdon Institute, Cambridge, UK)
03/10/14	**Francesca Peri**	(EMBL, Heidelberg, Germany)
03/10/14	**Darren Gilmour**	(EMBL, Heidelberg, Germany)
22/10/14	**Edward Arthur Curtis**	(Institute of Organic Chemistry and Biochemistry of the ASCR, v. v. i., Prague)
29/10/14	**Jiří Lukáš**	(Faculty of Health and Medical Sciences, University of Copenhagen, Denmark)
19/11/14	**Jaroslav Truksa**	(Institute of Biotechnology of the ASCR, v. v. i., Prague)
03/12/14	**Elena Taverna**	(Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany)

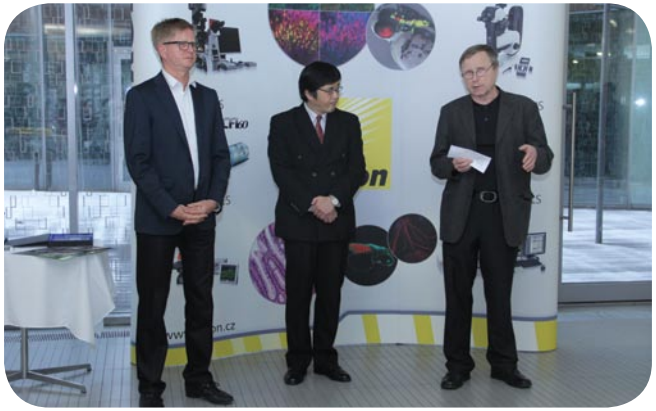
Highlights

CZ-OPENSCREEN opening ceremony

The opening ceremony of the new facility "CZ-OPENSCREEN: National Infrastructure for Chemical Biology" took place on September 24, 2013.
The Czech Minister for Education Youth and Sports Dr. Dalibor Štys (left), EU-OPENSCREEN coordinator Dr. Ronald Frank (middle), and director of Institute for Molecular Genetics of the Czech Academy of Sciences (IMG) Prof. Dr. Václav Hořejší (right) are cutting the ribbon for the official opening of the new CZ-OPENSCREEN infrastructure.
"CZ-OPENSCREEN: National Infrastructure for Chemical Biology" was established in pavilion V of the Institute of Molecular Genetics of the AS CR, v. v. i. and represents a new scientific centre supported by the "Operational Programme Prague - Competitiveness" (OPPC). The aim of this large investment project was to support basic and applied research in chemical biology and offer Open Access to academic researchers. The infrastructure is 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. Its mission is to create a national infrastructure for chemical biology comprised of the National compound collection and the database that enable identification of research tools and probes to be used in basic research and development of potential therapeutics. CZ-OPENSCREEN is a priority project within The National Roadmap of the Large Infrastructures and will serve as a National node in the ESFRI infrastructure EU-OPENSCREEN.

New Centre of Excellence for Super-resolution Microscopy

On January 21, 2014, company NIKON, in collaboration with the IMG, launched operation of a new Centre of Excellence for Super-resolution Microscopy. Super-resolution microscopy represents a revolutionary microscopic approach allowing observation of more minute details, namely in cell biology, than by using the so far employed conventional microscopy methods.
The Centre is part of the European Network of NIKON Centres of Excellence. Only five such centres exist in Europe (IMG Prague, ICFO Barcelona, Koki Budapest, UvA Amsterdam, Stockholm).
The NIKON Centre of Excellence functions as a partnership based on the knowledge exchange with the host institution, focused on a particular research area. The partnership provides scientists with access to cutting-edge advanced light microscopes and technical expertise NIKON. Company NIKON, on the other hand, receives a valuable feedback from scientist conducting top research using these instruments. The collaboration contributes to creating novel imaging solutions.



Acceptance in MERIL database

The Czech Centre for Phenogenomics was recognized in MERIL database (Mapping of the European Research Infrastructure Landscape) August 25, 2014.
The Czech Centre for Phenogenomics, part of the BIOCEV project and national node of ESFRI infrastructure Infrafrontier, has been recognized by European Science Foundation and included into MERIL database.
The MERIL database is an inventory of openly accessible research infrastructures (RIs) in Europe of more-than-national relevance across all scientific domains: from archives and statistical offices to biobanks, satellites and particle accelerators.

New instruments

CZ-OPENSCREEN

- Integrated robotic station for high-throughput screening
- Robotic integrated system for automated image analysis and label-free technology
- Integrated robotic system for compound storage and sample preparation
- Imaging microplate reader for real-time kinetic fluorimetric and luminescent measurements of second messengers
- Microplate beta counter
- Equipment for laboratory and tissue culture rooms

Centre for Model Organisms

- Zebrafish facility
- Super-resolution microscope STED (stimulated emission depletion) microscope (Leica TCS SP8 X STED3X WLL)
- Confocal microscope Lieca TCS SP8 confocal microscope

Czech-BioImaging Microscopy Centre

- Nikon AZ-100 zoom microscope with confocal head C2
  (microscope provided by Nikon, Nikon Centre of Excellence run as the part of the facility)
- N-SIM structured illumination microscope
  (microscope provided by Nikon, Nikon Centre of Excellence run as the part of the facility)

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

Operational Programme Research and Development for Innovations

More than 25,000 m² floor space, equipped with state-of-the-art equipment and technologies, 600 researchers and students included in more than 50 research teams and 5 programmes. Biotechnology and Biomedical Centre in Vestec (BIOCEV) is a new European scientific centre of excellence, whose outputs will lead to a better quality of life and knowledge economy.

The first BIOCEV research programme (Functional Genomics) was launched in August 2012 and is headed by Assoc. Prof. Radislav Sedláček, Ph.D. R. Sedláček is also the Head of the Czech Centre for Phenogenomics (CCP, also known as the ´Mouse Clinic´), the largest comprehensive rodent research infrastructure in the Central Europe and BIOCEV´s core 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. Transgenic and gene 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 drugs and toxins, thus helping to answer fundamental issues in basic and applied research.

The following research programmes of BIOCEV are Cellular Biology and Virology, Structural Biology and Protein Engineering, Biomaterials and Tissue Engineering, and Development of Diagnostic and Therapeutic Procedures. More details on the research programmes can be found at the project website **http://www.biocev.eu/en/research-programme/**

BIOCEV

Pillars

The uniqueness of the project lies in its balanced combination of top research integrated in five programmes, education and training of undergraduate and graduate students, and intensive cooperation with the commercial sector. Led by renowned experts, BIOCEV research teams have access to cutting-edge technologies and are an active part of major European groupings such as INFRAFRONTIER, Euro-BioImaging, or INSTRUCT.

Implementation of the BIOCEV project is a collaborative effort 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). The project 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 the Institute of Molecular Genetics.

Project Schedule

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 in July 2015) 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 foundation stone ceremony was organized on October 7th, 2013. About 10 months later, on August 27th, 2014, the BIOCEV main construction site was completed. Commencement of the trial operational phase of the Centre is expected in Autumn 2015.

From the left: Václav Hořejší, Director of IMG, H.E. Jan Thompson, Ambassador of Great Britain to the Czech Republic, and Pavel Martásek, Director of BIOCEV

Nearly 25,500 m² of new laboratories and other working space will accommodate 600 employees, of which 450 will be scientists, including nearly two hundred students of graduate and postgraduate programmes from the 1st Faculty of Medicine and the Faculty of Science at Charles University.

From the left: Václav Hampl, Rector of Charles University in Prague, Vladimír Mareček, Vice-president of the Academy of Sciences of the Czech Republic, Jiří Rusnok, Prime Minister, Miroslava Kopicová, Member of the R&D Council of the Czech Republic, Martin Holcát, Minister of Health, Dalibor Štys, Minister of Education, Youth and Sports, Tibor Švec, Mayor of Vestec

Awards & Honours

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

2013

Jiří Bártek – František Běhounek Award for excellent representation of the Czech Republic in European Research and Development
Jiří Bártek, Václav Hořejší – Silver Memorial Medal of the Senate of the Parliament of the Czech Republic for their scientific achievements
Romana Mikyšková – Milan Pospíšil Award for 2013 for an original report in the field of innate and anti-tumour immunity (Czech Immunological Society)
Jana Písačková – Heart of Europe Bio-Crystallography Meeting 2013 Award for the best presentation
Václav Pačes – Award of the Prague Mayor for the contribution to organization of conferences in Prague

2014

Petr Svoboda – Neuron Fund Prize in the field of medicine
Jiří Hejnar and his team – Prize of the Academy of Sciences of the Czech Republic
Jan Kosla – Prize for an Outstanding Paper in the Field of Oncology published in 2013, awarded by the League Against Cancer
Ondřej Štěpánek – Prize for the Best Paper by a Young Immunologist in 2013 (Czech Immunological Society)
Matyáš Flemr – Discovery Award from company Novartis for basic research in biomedicine

Seminars & Conferences

www.img.cas.cz/events/

Conferences and Courses

2013

07/06	6th IMG PhD Conference
4-15/11	37th Advances in Molecular Biology and Genetics
10-14/11	Course "Microscopy Methods in Biomedicine"
18-22/11	Course "Transmission Electron Microscopy in Life Sciences"
28-29/11	Symposium "Visualizing the Invisible in Cell Biology"
13/12	Annual IMG Conference

2014

24-28/03	Elements of Science
1/04	Genome Editing using Programmable Nucleases
19-23/05	Course "Processing and Analysis of Microscopic Image in Biomedicine"
20/06	7th IMG PhD Conference
28/08	Animal welfare and the 3Rs under directive 2010/63/EU meeting
7-12/09	18th International Microscopy Congress
3-14/11	38th Advances in Molecular Biology and Genetics
10-14/11	Course "Microscopy Methods in Biomedicine"
12-14/11	Practical Course on Fish Transgenesis
28/11	Annual IMG Conference
10-12/12	Advanced Techniques in Immunohistochemistry and Transgenesis

Regular weekly Institute seminars – IMG speakers

2013

Jan Dobeš
Jan Benada
Mária Dzúr-Gejdošová
Iva Polakovičová
Jolana Turečková
Jana Písačková
Ondřej Svoboda
Yahya Sohrabi
Aleš Drobek
Libor Macůrek
Jan Bražina
Zuzana Hájková
Denisa Kovářová
Pavel Otáhal
Alena Hájková
Jan Kosla
Lucie Tůmová
Samira Hozeifi

2014

Petr Svoboda
Jan Pačes
Jana Balounová
Jan Mašek
Petr Flachs
Monika Bambousková
Alžběta Kalendová
Trevor Epp
Petr Pachl
Aleš Drobek
Jana Konířová
Vadym Sulimenko
Lenka Kyjacová
Libor Macůrek
Igor Grekov
Jiří Hejnar
Lucie Láníková
Jan Dobeš

PhD Programme

www.img.cas.cz/phd

Students represent a significant element in our scientific community; the presence of about 100 PhD students (20 % international from both EU and non-EU countries) considerably enriches the atmosphere at the Institute and strongly contributes to its scientific output. Therefore, one of our priorities is to offer an appealing PhD programme that will attract the best students and 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 Kozmik, David Staněk, Petr Svoboda) and student representatives (Michaela Liegertová, Eva Šimková 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. PhD students routinely present in English during lab meetings, journal clubs, and during institutional weekly seminars, which are almost exclusively given by PhD students.
Students apply to the programme through an on-line application at http://www.img.cas.cz/phd, where all the open PhD positions and relevant deadlines are posted. This makes our PhD programme better accessible also to students abroad, and the PhD community at our Institute is becoming more and more international. In each of the years 2013 and 2014, about 30 candidates were selected 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. In the end, 16 students were recruited during the PhD interview procedure in 2013 and 23 in 2014.

We also aim to foster extracurricular training of our PhD students. Since 2010, we have organized a "Welcome Weekend" for the new PhD students, 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.

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

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 6 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, for the period of two years. The project was financed by the European Social Fund (ESF). The project was concluded in October 2014.

The main goal of the kindergarten is to enable parents to easily return to work. It considerably alleviates the current problem with insufficient capacity of state-run kindergartens.
The kindergarten introduces many new activities that promote comfort and professionalism for all parents and children. The kindergarten particularly supports individual approach to the child and very close cooperation with its family. It promotes active participation of the parents in the kindergarten activities and events. It aims at mutual and open communication and collaboration creating a partnership between the kindergarten and the family.
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.

OPPA PRAHA PRAGUE PRAGA PRAG EVROPSKÁ UNIE

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Teaching and Courses

Innate Immunity, Faculty of Science, Charles University in Prague (D. Filipp)

Advances in Immunology, Faculty of Science, Charles University in Prague (D. Filipp, J. Dobeš)

Pharmacology, Second Faculty of Medicine, Charles University in Prague (J. Blahoš)

Immunity to Infection, Third Faculty of Medicine, Charles University in Prague (M. Lipoldová)

Advances in Molecular Immunology, Third Faculty of Medicine, Charles University in Prague (M. Lipoldová)

Fundamentals of Molecular Biology, Faculty of Biomedical Engineering, Czech Technical University in Prague (M. Lipoldová)

RNA structure and Function, Faculty of Science, Charles University in Prague (D. Staněk /N. Bieberstein)

Molecular Biology of Cancer, Faculty of Science, Charles University in Prague (V. Kořínek)

Molecular Genetics of Mammalian Organism, Faculty of Science, Charles University in Prague (J. Forejt)

Strategy and Tactics of Grant Proposal, Faculty of Science, Charles University in Prague (Petr Dráber)

Molecular Mechanisms of Apoptosis, Faculty of Science, Charles University in Prague (L. Anděra)

Immunology (basic course), Faculty of Science, Charles University in Prague (V. Hořejší, T.Brdička)

Epigenetics, Faculty of Science, Charles University in Prague (annually)/ University of Southern Bohemia, České Budějovice (bi-annually) (P. Svoboda)

Model Organisms in Developmental Biology, Faculty of Science, Charles University in Prague (Z. Kozmik)

Three-Dimensional Structure Solution of Macromolecules, Faculty of Science, Charles University in Prague (J. Brynda and P. Řezáčová)

Genome Integrity in Cancerogenesis and Ageing, Faculty of Science, Charles University in Prague (J. Bártek, with participation of L. Macůrek)

Structure and Function of the Cytoskeleton, Faculty of Science, Charles University in Prague (Pavel Dráber)

Bachelor Theses 2013

Jan Bartůněk	Methods of biomedical research in mapping genes in an experimental model in health and disease (Supervisor: Marie Lipoldová, Taťána Jarošíková, Faculty of Biomedical Engineering, Czech Technical University in Prague)
Tereza Pokorná	Methods of genetic engineering for identification of the genes that modify response to parasite Leishmania major (Supervisor: Marie Lipoldová, Taťána Jarošíková, Faculty of Biomedical Engineering, Czech Technical University in Prague)
Karin Heyduková	Methods of biomedical research in mapping genes in an experimental model – fine mapping of the Lmr locus on chromosome 5 (Supervisor: Marie Lipoldová, Taťána Jarošíková, Faculty of Biomedical Engineering, Czech Technical University in Prague)
Marta Patrná	Methods of biomedical research in mapping genes in an experimental model – fine mapping of the Lmr locus on chromosome 10 (Supervisor: Marie Lipoldová, Taťána Jarošíková, Faculty of Biomedical Engineering, Czech Technical University in Prague)

Bachelor Theses 2014

Ondřej Dostál	Methods of genetic engineering in mapping genes that modify organ size (Supervisor: Marie Lipoldová, Taťána Jarošíková, Faculty of Biomedical Engineering, Czech Technical University in Prague)
Zuzana Ezrová	Genes participating in response to Leishmania major revealed by targeted mutation (Supervisor: Marie Lipoldová, Faculty of Science, Charles University in Prague)
Šimon Borna	Transmembrane adaptor proteins (Supervisor: Václav Hořejší, Faculty of Science, Charles University in Prague)

Diploma Theses 2013

Dalibor Miklík	Functional genome analysis using the retroviral integration sites permissive for provirus expression in human cells (Supervisor: Jiří Hejnar, Filip Šenigl, Faculty of Science, Charles University in Prague)
Daniel Matějů	Functional analysis of hPrp8 mutations linked to retinitis pigmentosa (Supervisor: Zuzana Cvačková, Faculty of Science, Charles University in Prague)
Martin Peterka	Characterization of TRAIL-induced, receptor-specific signalling in cancer cells (Supervisor: Ladislav Anděra, Faculty of Science, Charles University in Prague)
Terezie Imrichová	The response of metastatic prostate cancer cell lines to genotoxic stress (Supervisor: Zdeněk Hodný, Faculty of Science, Charles University in Prague)
Klára Kotlabová	The role of Src-family kinases in the immunological synapse of antigen-presenting cells (Supervisor: Tomáš Brdička, Faculty of Science, Charles University in Prague)

Diploma Theses 2014

Martina Benešová	Human endogenous retrovirus ERVWE1: transcriptional activation and modifications of promoter DNA methylation (Supervisor: Kateřina Trejbalová, Faculty of Science, Charles University in Prague)
Jan Valečka	Identification of a new mechanism of Lck regulation via its C-terminal sequence (Supervisor: Dominik Filipp, Faculty of Science, Charles University in Prague)
Matouš Vobořil	Aire-expressing cells in immune peripheral tissues (Supervisor: Dominik Filipp, Faculty of Science, Charles University in Prague)
Michaela Dvořáková	Molecular aspects of Cannabinoid receptor 1 signalling (Supervisor: Jaroslav Blahoš, Faculty of Science, Charles University in Prague)
Adriana Roithová	Transport of U2 snRNA to Cajal bodies (Supervisor: David Staněk, Faculty of Science, Charles University in Prague)
Oľga Babošová	Characterization of novel synthetic inhibitors and activators of the Wnt signalling pathway (Supervisor: Vladimír Kořínek, Georg-August-Universität, Gottingen, Germany)
Gita Nováková	Characterization of the role of senescence in the induction and regulation of cancer cell death (Supervisor: Ladislav Anděra, Faculty of Science, Charles University in Prague)
František Pešina	Comparison of the induction and regulation of autophagy in proliferating and senescent cancer cells (Supervisor: Ladislav Anděra, Faculty of Science, Charles University in Prague)
Veronika Machalová	The role of 5-azacytidine in therapy of myelodysplastic syndrome (Supervisor: Zdeněk Hodný, Faculty of Science, Charles University in Prague)
Jan Valášek	A potential role of DAXX in cell cycle arrest and cellular senescence (Supervisor: Hana Hanzlíková, Faculty of Science, Charles University in Prague)

PhD Theses 2013

Jan Lukáš	Molecular mechanisms of Wnt signalling in mammalian cells (Supervisor: Vladimír Kořínek, Faculty of Science, Charles University in Prague)
Viktor Bugajev	Identification and functional characterization of signalling microdomains important for mast cell activation (Supervisor: Petr Dráber, Faculty of Science, Charles University in Prague)
Vladimíra Horová	Mechanism, regulation and use of TRAIL-induced apoptosis in cancer cells (Supervisor: Ladislav Anděra, Faculty of Science, Charles University in Prague)
Jana Nejepínská	The effects of long double-stranded RNA expression in mammalian cells (Supervisor: Petr Svoboda, Faculty of Science, Charles University in Prague)
Iryna Kozmiková	Bmp signalling in the evolution of chordate axial patterning (Supervisor: Zbyněk Kozmik, Faculty of Science, Charles University in Prague)
Jan Kosla	Molecular mechanisms of fibroblastoid cell phenotype transitions: dedifferentiation of myofibroblasts and influencing of invasiveness and metastasis of sarcoma (Supervisor: Michal Dvořák, Faculty of Science, Charles University in Prague)
Vladimír Čermák	Regulation of transcription by proteins of the Early growth response and Myb families (Supervisor: Michal Dvořák, Faculty of Science, Charles University in Prague)
Sukriye Yildirim	Myosin-PIP2 interaction in the cell nucleus (Supervisor: Pavel Hozák, Instanbul University, Turkey)
Tomáš Venit	Myosin 1c isoforms and their functions in the cell nucleus and in the cytoplasm (Supervisor: Pavel Hozák, Faculty of Science, Charles University in Prague)

PhD Theses 2014

Jana Balounová	Toll-like receptors and myeloid cells in development and disease (Supervisor: Dominik Filipp, Faculty of Science, Charles University in Prague)
Yahya Sohrabi	Leishmania tropica: immunopathology and genetic control (Supervisor: Marie Lipoldová, Third Faculty of Medicine, Charles University in Prague)
Samira Hozeifi	Regulation of alternative splicing via chromatin modifications (Supervisor: David Staněk, Faculty of Science, Charles University in Prague)
Eva Kozáková (Dušková)	Role of promoter in the regulation of alternative splicing (Supervisor: David Staněk, Faculty of Science, Charles University in Prague)
Michaela Krausová	Molecular mechanisms and components controlling the Wnt signalling pathway output (Supervisor: Vladimír Kořínek, Faculty of Science, Charles University in Prague)
Lucie Janečková	Wnt signalling in intestinal homeostasis and tumorigenesis (Supervisor: Vladimír Kořínek, Faculty of Science, Charles University in Prague)
Iva Polakovičová	Multiple regulatory roles of the transmembrane adaptor protein NTAL in gene transcription and mast cell physiology (Supervisor: Petr Dráber, Faculty of Science, Charles University in Prague)
Petr Pachl	Structural and inhibition study of 5'(3')-nucleotidases (Supervisor: Jiří Brynda, Institute of Chemical Technology, Prague)
Martin Košař	Mechanisms of replicative, drug- and oncogene-induced cellular senescence (Supervisor: Jiří Bartek, First Faculty of Medicine, Charles University in Prague)
Kamila Burdová	Molecular mechanisms underlying maintenance of genome stability (Supervisor: Pavel Janščák, Faculty of Science, Charles University in Prague)
António Pombinho	High-throughput screening for the discovery of small molecules modulating cell fate (Supervisor: Petr Bartůněk, Faculty of Science, Charles University in Prague)
Alžběta Kalendová	Functions of actin and myosin 1c in the cell nucleus and in the cytoplasm (Supervisor: Pavel Hozák, Faculty of Science, Charles University in Prague)
Anatoly Philimonenko	Development of ultrastructural methods and their application in studies of the cell nucleus (Supervisor: Pavel Hozák, Faculty of Science, Charles University in Prague)

Our Location

www.img.cas.cz/contact/how-to-find-us/

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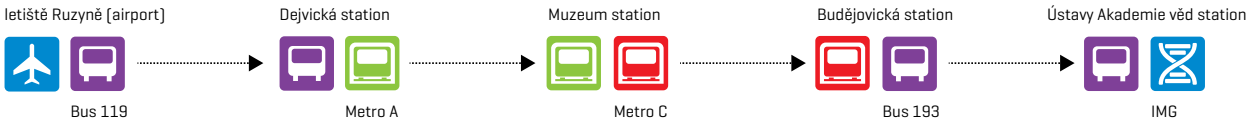
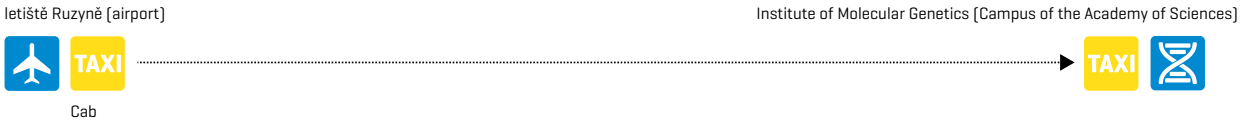
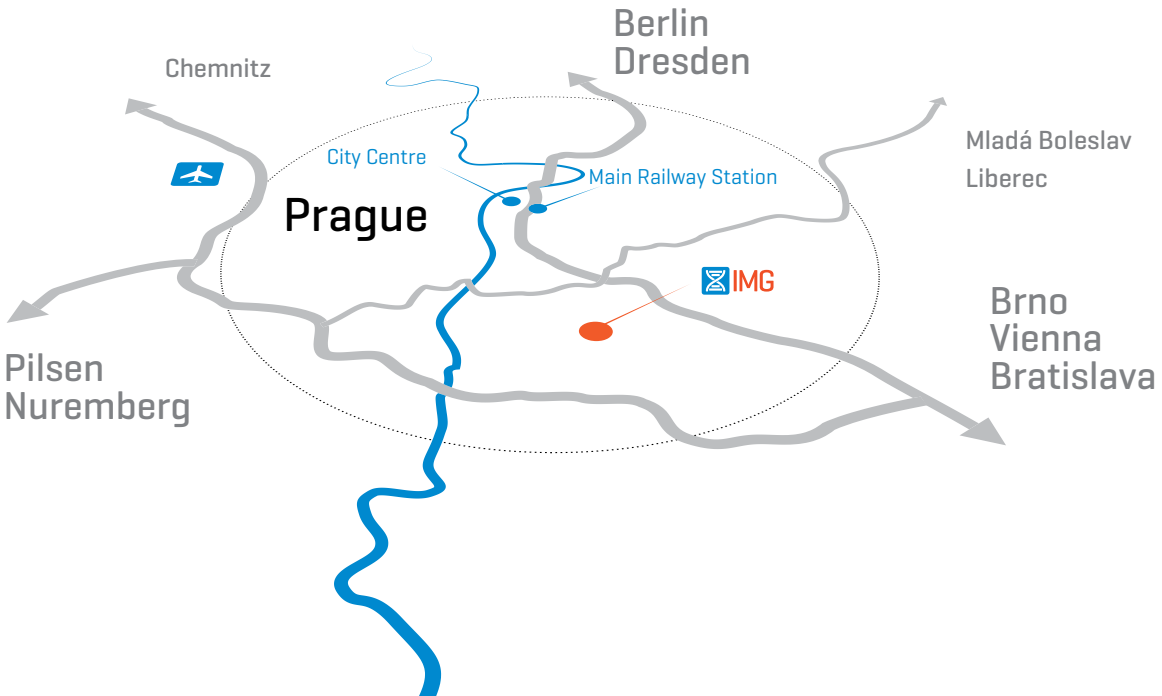
Prague 4, Czech Republic







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