IMG

Institute of Molecular Genetics of the Czech Academy of Sciences

60

YEARS ANNIVERSARY

1962 - 2022

ANNUAL REPORT 2020 - 2022

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FOREWORD FROM IMG DIRECTOR

Dear Colleagues,

The three-year Scientific Report of the Institute of Molecular Genetics of the Czech Academy of Sciences (IMG) summarizes the most remarkable results and activities we achieved in the years 2020-2022. In contrast to previous years, our work was markedly affected by the coronavirus pandemic, which stroke us in several waves in 2020, 2021 and 2022. The Covid pandemic afflicted us with many restrictions aimed at slowing down the spread of coronavirus infection. In addition, our employees were involved in testing samples for the presence of SARS-Cov-2, and they actively participated in raising awareness in the fight against the infection spread. In 2022 we were also affected by a dramatic increase in energy prices.

Scientific research continued in 2020-2022 in research groups and four large national research infrastructures (Czech-BioImaging, CZ-OPENSCREEN and ELIXIR CZ in Krč, and Czech Centre for Phenogenomics in Vestec). Many changes have taken place in research groups in that period. In 2020, the Service Laboratory of Functional Genomics and Bioinformatics and the Genomics and Bioinformatics Division merged into the Laboratory of Genomics and Bioinformatics and a new group leader was appointed based on the competitive selection process. Furthermore, a new Head of the Laboratory of Genome Integrity was selected. Finally, the Laboratory of Molecular and Cellular Immunology was closed. In 2021, in BIOCEV, two laboratories were closed, the Laboratory of Biology of the Eye and the Department of Epigenetics of the Cell Nucleus, and in Krč, a new Proteomics Service Laboratory was established. In 2022, several laboratories moved to different categories: the Laboratory of Haemato-Oncology moved from the Junior Group to the Senior Group; the Laboratory of Genome Dynamics moved from the Guest Group to the Junior Group; the Laboratory of Leukocyte Signalling moved from the Senior Small Group to Senior Group, and the Laboratory of Cell Motility moved from IMG Fellows to Junior Group. Furthermore, a new Junior Group, Laboratory of Developmental Mechanobiology (No. 10), was established as a result of the competitive selection process for a new group leader. That year, the IMG part of the Laboratory of Structural Biology in Dejvice was closed, and all people moved into the Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences.

At the end of 2022, our Institute had 535 employees (471 full-time equivalents). About two-thirds of them worked in Krč, one-third in Vestec as a part of the project BIOCEV, and a minor part worked at the Koleč farm. The Institute comprised 281 employees working in research and research service groups, 27 in research services, 168 in research infrastructures, 51 in administration and technical services and 8 in the director's office. We have about 100 Ph.D. students.

In 2021, an evaluation of our Institute in the period 2015-2019 was finalized with predominantly very positive assessments at the level of the Institute performance and research teams quality. In 2022, we introduced a new IMG website that summarizes important research discoveries we obtained in 2020 – 2022. Our results in this time period were described in 418 publications and presented at numerous international conferences and symposia, including those organized by IMG researchers. The activity of our research groups also resulted in numerous application outputs and patents, which in many cases reflected cooperation with the business sphere and contributed considerable financial means to our Institute budget. To facilitate the implementation of the application results, the Institute reinforced the Centre of technology transfer and continued collaboration with the i&i company. The applied research at IMG was also supported by the TACR GAMA 2 projects.

At that time, as in previous years, we obtained the major part of the funding (~60 %) that sustained our basic research tasks in the form of specific grants from various grant agencies and other funding providers. However, the dependence on short-term grants (usually for about three years) caused that, on average, each group and infrastructure participated in more than four projects. This resulted in a sizeable bureaucratic burden for principal investigators. To help researchers with grant agendas and manage grant

On 14 December 2020, six directors of institutes of the Academy of Sciences and the rector of Charles University signed a new partnership agreement of the BIOCEV Centre.

inspections, we have strengthened the grant group and integrated it into the Economy Division in 2022.

As in previous years, we paid a lot of attention to the BIOCEV project in Vestec, of which our Institute was the guarantor for the period of the compulsory sustainability phase (2016 – 2020). All project partners agreed to continue participating in the BIOCEV project after 2020 as a centre of excellent research in cooperation of two Charles University faculties and six Academy institutes, of which the Institute of Biotechnology (IBT) represents the only partner residing in Vestec; all other partners have their detached sites in this locality. Considerable attention of the BIOCEV administrative team and BIOCEV Board was focused on preparing the new Partner Agreement, defining terms of operation of BIOCEV departments and units after 2020. This Agreement was signed on December 14, 2020 by all seven statutory representatives of the project BIOCEV partners.

During 2020, we obtained legal analyses for the transfer, in the public interest (without tax burden), of a part of the main building and energy centre, constructed within the framework of project BIOCEV, from IMG to IBT. Starting from January 1st, 2021, the BIOCEV administrative team was transferred to IBT as well.

IMG administrative departments play an essential role in securing conditions for the research of our entire Institute. A major task for the administrative team in 2020 was implementing the new financial information system from the company Magion system, a.s. The task was successfully completed, and since the beginning of 2021, the Magion system has been used. Our administrative and technical departments, including the Administrative Team, Economy Department, Building Administration, and IT Department, provided performance at the required level. Most of the research groups at our Institute also rely on animal breeding facilities situated in Krč, Vestec and Koleč. Despite the problems associated with the coronavirus pandemic, these facilities showed trouble-free operation. We also continued renovations and performed numerous repairs in Koleč, Krč and BIOCEV, where we built a lab for work with infectious material at the BSL3 level.

Meeting to celebrate the 60th anniversary of IMG on 26 May 2022. From left to right: former Director Václav Hořejší, Secretary of all three directors Leona Krausová, former IMG Director and former Chairman of the Academy of Sciences of the Czech Republic Václav Pačes and current IMG Director Petr Dráber.

Despite Covid-19 restrictions, we multiplied efforts to popularise our activities and results, including those related to the covid-19 disease. Great attention was paid to the organization of both Ph.D. programs and Ph.D. conferences in the demanding conditions of the pandemic. In an effort to create better interaction of biomedically oriented institutions on the campus of biology institutes in Krč and in the BIOCEV Centre in Vestec, a new website offering the PhD study positions at all participating institutions has been created. As part of the yearly promotions, we organized IMG Open Doors Days. Many additional Institute activities have been recorded on our web pages, Facebook, Instagram, Twitter and LinkedIn.

In 2022, we commemorated the 60th anniversary of our Institute, which dates back to the founding of the Institute of Experimental Biology and Genetics of the Czechoslovak Academy of Sciences in 1962, whose first director was Milan Hašek. On this round anniversary of the institute, our former director and chairman of the Czech Academy of Sciences Václav Pačes gave an interesting lecture in the framework of the all-institutional assembly associated with the party.

In conclusion, in 2020-2022, our Institute continued its scientific excellence and importance, as documented in this Annual Report. I am convinced that IMG, based on the people, equipment, services, presence of large national research infrastructures, animal facilities, and administrative team, has all the necessary prerequisites for further development and essential contributions to the basic knowledge in the field of molecular cell biology and genetics, and also in transfer of new scientific findings and tools to their practical applications. I am convinced that IMG, based on the people, equipment, services, presence of large national research infrastructures, animal facilities, administrative team and ability to obtain support for our research from prestigious grant agencies has all the necessary prerequisites for further development and essential contributions to the basic knowledge in the field of molecular cell biology and genetics, and also in transfer of new scientific findings and tools to their practical applications.

January 15, 2023

Petr Dráber

INSTITUTE MANAGEMENT

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Director of the Institute

Prof. David Staněk, Ph.D.
Deputy Director

Martin Polák, M.Sc.
Deputy Director for Administration

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Claudio Sunkel
Institute for Molecular and Cell Biology,
Porto, Portugal

BRIEF HISTORY

The history of our Institute ensues from the Department of Experimental Biology and Genetics of the Institute of Biology of the Czechoslovak Academy of Sciences, headed since 1953 by Milan Hašek, co-discoverer of immunological tolerance.

In 1962, the Institute of Experimental Biology and Genetics of the Czechoslovak Academy of Sciences (IEBG) was founded, with Milan Hašek as its Director until 1970. The sixties of the last century mark, without doubt, the most memorable chapter of the Institute – the "Czechoslovak immunogenetic school" was born at that time, represented besides Hašek by such names as Pavol and Juraj Iványi, Jan Klein, Tomáš Hraba, Ivan Hilgert, Věra Hašková, Alena Lengerová, and others. It is generally known that Milan Hašek came close to the Nobel Prize (for the discovery of immunological tolerance, it was awarded to P. Medawar and M. Burnet); Pavol Iványi contributed significantly to the experiments

whose results later brought the Nobel Prize to Jean Dausset; Jan Klein, after emigration to the US, in the seventies became probably the most eminent immunogeneticist worldwide (co-discoverer of the fundamental immunological significance of MHC proteins). During this period, great attention at IEBG was also paid to the development of the worldwide priority research of retroviruses (Jan Svoboda).

In the years 1964-2006, the major part of the Institute had its site in the building belonging to the Institute of Organic Chemistry and Biochemistry of the Czechoslovak Academy of Sciences (later Academy of Sciences of the Czech Republic) (IOCB) located at the address Flemingovo náměstí, Prague – Dejvice, and the minor part was situated in the complex of biological institutes of the Academy in Prague – Krč. Another important part of the Institute is the breeding and experimental farm in Koleč (about 20 km from Prague).

The end of the "Prague Spring" after August 1968 marked the end to this famous era – many promising young scientists had emigrated (and were very successful at their new institutions abroad), Milan Hašek was removed from the post of Director of the Institute, and contacts with other countries were drastically limited.

After a short period during which the Institute was headed by Karel Heyberger, Prokop Málek served as Director of IEBG in 1970-1977.
In 1976, IEBG was joined with several biochemical laboratories of IOCB and renamed Institute of Molecular Genetics of the Czechoslovak Academy of Sciences (IMG). The post of IMG Director was conferred on Josef Říman (later appointed as President of the Czechoslovak Academy of Sciences for many years), who stayed in this position until 1991. Since that time, molecular biology has become the main topic of the Institute, but additional, traditional orientations have remained (immunogenetics, retrovirology, tumour immunology); these, however, also have gradually transferred to the molecular level. Other achievements from 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 continued the trend of enhancing the molecular biology approaches to the traditional as well as newly introduced topics. The Institute was first headed by Jan Svoboda (1991-1999) and then by Václav Pačes (1999-2005). In 2004, in the Krč site of the Academy, construction of a new building was started to house the Institute, in which since 2007 (for the first time in its history) a large majority of the Institute employees have finally gathered. After V. Pačes had been elected President of the Academy of Sciences of the Czech Republic, in 2005, Václav Hořejší became Director of the Institute.

In 2015 a new building of centre BIOCEV was completed and fully equipped in a nearby township Vestec, where several IMG research groups and National Research Infrastructure "Czech Centre of Phenogenomics" is located. In the same period, two other research infrastructures started operating in Krč, namely, CZ-OPENSCREN and CZECH BIOIMAGING hosted by the Krč part of IMG. In May 2017, Petr Dráber became IMG Director. At that time, the Institute included the central part on the Krč campus and three detached sites, one in Vestec (as part of the BIOCEV project), one in Dejvice and one in Koleč. In 2022, based on agreement between IMG and IOCB, the group in Dejvice was transferred to the IOCB.

STATISTICS

The statistics correspond to the situation at the end of 2022

PEOPLE

255 Employees in scientific departments

221 Employees in service departments

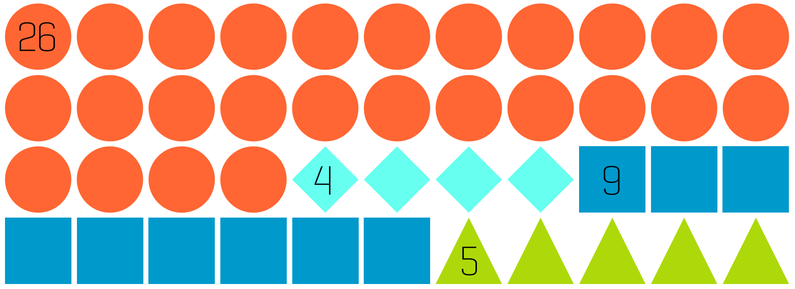
59 Administrative staff

535 TOTAL 193 employees located in BIOCEV

107 Ph.D. students

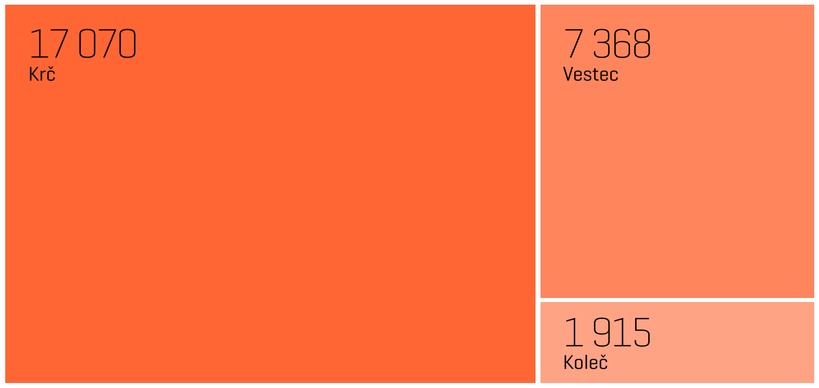
GROUPS AND INFRASTRUCTURES

Research groups · Large research infrastructures · Service groups · Administrative services



SPACE

Total floor area / m2



PAPERS

Journal publications
2020 – 2022

418

RESEARCH GROUPS

DEVELOPMENTAL MECHANOBIOLOGY

Quantitative live-imaging, cytoskeletal dynamics, nematode genetics, biophysics, evolutionary development (evo-devo)

Teije Corneel Middelkoop

The shape of an animal arises in a species-specific, step-wise fashion during embryonic development. During this sequence of events, collectively referred to as 'embryo morphogenesis', the embryo constantly remodels its shape. Our lab is interested in the force-generating mechanisms that drive these shape changes.

We aim at addressing the following questions:

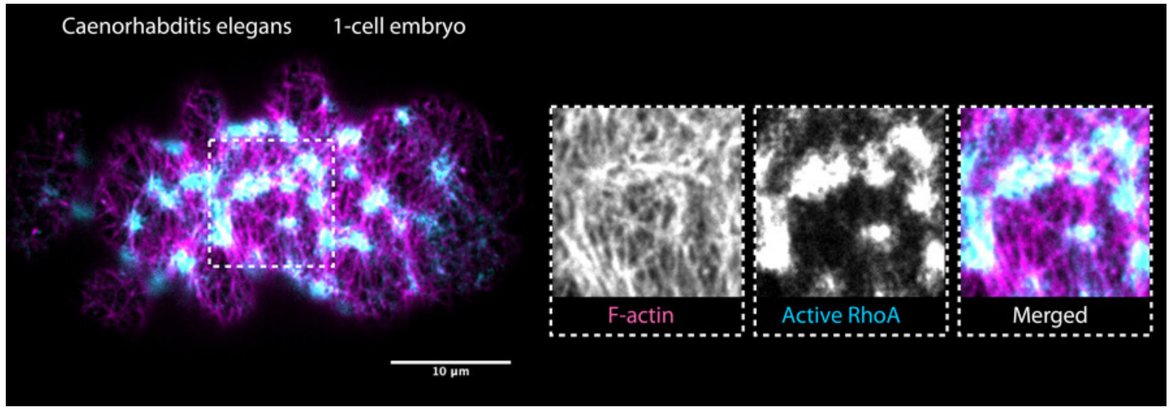
1. How do molecular-scale forces arise in developing embryos?
2. How do molecular-scale forces drive embryonic shape changes?
3. How do these force-generating mechanisms change on evolutionary time scales?

Our research operates at the intersection between cell biology, evolutionary developmental biology (evo-devo) and biophysics, and employs quantitative methodology from all these disciplines.

The forces that drive embryo morphogenesis are generated by the cytoskeleton of embryonic cells and mainly arise in the actomyosin cortex: a dense two-dimensional network of cross-linked actin polymers residing directly underneath the plasma membrane (see figure 1). By pulling and twisting, actin polymers, myosin motors and numerous accessory proteins can generate active forces within the cortical layer. Tight spatiotemporal regulation of these molecular-scale forces in developing embryos ultimately results in cellular-scale shape changes required for embryo morphogenesis.

We use Caenorhabditis elegans and related nematode species to study how molecular forces generated within the actomyosin layer give rise to morphogenetic shape changes in early embryos. To this end, we combine the strength of C. elegans genetics with time-lapse imaging of early embryos (both at high-resolution and at super-resolution), quantitative image analysis and biophysical modelling.

In the picture: 1. Grootel Jacobus van | 2. Sýkorová Denisa | 3. Akandwanaho Allan | 4. Middelkoop Teije Corneel



Actomyosin cortex of a one-cell Caenorhabditis elegans embryo. Actomyosin is visualized using fluorescent markers for filamentous actin (F-actin, magenta) and regions where active forces and torques are generated (Active RhoA, cyan). Bottom panels show a magnification of the region marked in the top panel.

Selected publications:

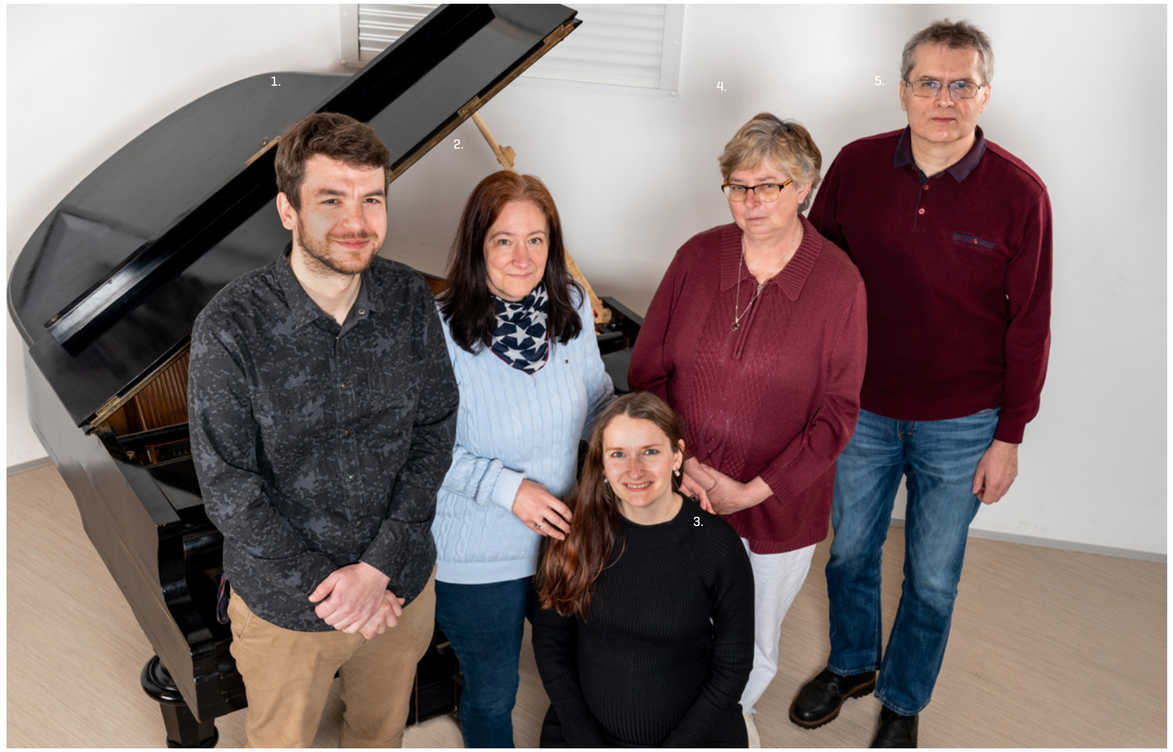
1. Middelkoop TC, Garcia-Baucells J, Quintero-Cadena P, Pimpale L, Yazdi S, Sternberg P, Gross P, Grill SW*. CYK-1/Formin activation in cortical RhoA signaling centers promotes organismal left-right symmetry breaking. PNAS, 118, 20, e2021814118, (2021).
2. Pimpale LG, Middelkoop TC, Mietke A, Grill SW*. Cell lineage-dependent chiral actomyosin flows drive cellular rearrangements in early Caenorhabditis elegans development. Elife 9, e54930, (2020).

LABORATORY OF

IMMUNOLOGICAL AND TUMOUR MODELS

Experimental cancer therapy, tumour immunology, murine models, JAK/STAT signalling, cellular senescence

Milan Reiniš



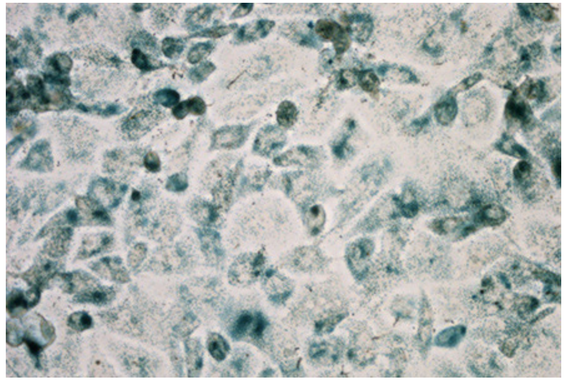
In the picture: 1. Novotný Ondřej | 2. Mikyšková Romana | 3. Sapega Olena | 4. Turečková Renáta | 5. Reiniš Milan

Our long-term research interest lies in interactions between tumour cells and the immune system, as well as the impacts of anti-tumour chemo- and immunotherapies on these interactions. We have been focused on the mechanisms by which tumour cells can escape from immune responses, such as MHC class I downregulation on tumour cells or mechanisms of the immune suppression development in the tumour microenvironment.

At present, we concentrate on the impacts of genotoxic stress and cellular senescence induction by chemotherapeutic agents or cytokines on the crosstalk between tumour cells and the immune system. Cellular senescence represents an important barrier against cancer development. However, the presence of senescent cells, or cells in a genotoxic stress in general, can influence wthe microenvironment in different ways, and it can also have detrimental effects on the tumour growth and anti-tumour immunity.

JAK/STAT signalling pathways play important roles in the processes mentioned above. Recently, we have concentrated on the role of STAT1 in cellular stress/senescence induction. Further, we suppose that STAT3 signalling pathway inhibition can be an important tool for elimination of the negative effects of chemotherapy, and it can also increase its efficacy and eliminate immune suppression. Therefore, we study novel and existing STAT3 inhibitors and their potential clinical usage in murine preclinical models.

In collaboration with several partners, we test novel immune and chemotherapeutic approaches, using syngeneic murine models. We use tumours induced by syngeneic tumour cell transplantation, as well as transgenic mice as orthotopic models that develop spontaneous tumours. We also employ experimental models for minimal residual tumour disease after surgery or chemotherapy. Indeed, we are open to more future collaborations and contract research.



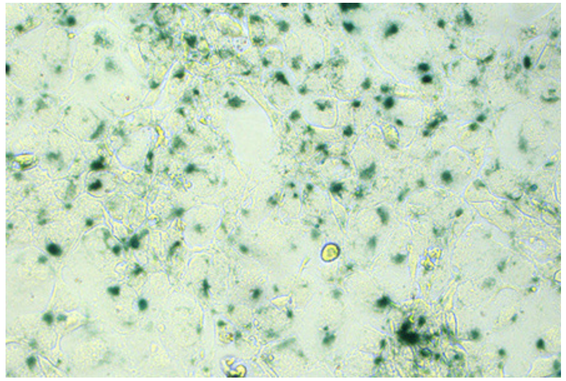


Image of senescent cells stained with b-galactosidase

Selected publications:

1. Mikyskova R, Sapega O, Psotka M, Novotny O, Hodny Z, Balintová S, Malinak D, Svobodova J, Andrys R, Rysanek D, Musilek K, Reinis M*. STAT3 inhibitor Stattic and its analogues inhibit STAT3 phosphorylation and modulate cytokine secretion in senescent tumor cells. Mol Med Rep Accepted.
2. Koncošová M, Rumlová M, Mikyšková R, Reiniš M, Zelenka J, Ruml T, Kirakci K*, Lang K: Avenue to X-ray-induced photodynamic therapy of prostatic carcinoma with octahedral molybdenum cluster nanoparticles. J Mater Chem B 2022 10:3303-3310.
3. Grusanovic S, Danek P, Kuzmina M, Adamcova MK, Burocziova M, Mikyskova R, Vanickova K, Kosanovic S, Pokorna J, Reinis M, Brdicka T, Alberich-Jorda M*. Chronic inflammation decreases HSC fitness by activating the druggable Jak/Stat3 signaling pathway. EMBO Rep 2023 24(1):e54729.
4. Oleksak P, Psotka M, Vancurova M, Sapega O, Bieblova J, Reinis M, Rysanek D, Mikyskova R, Chalupova K, Malinak D, Svobodova J, Andrys R, Rehulkova H, Skopek V, Ngoc Lam P, Bartek J, Hodny Z*, Musilek K*. Design, synthesis, and in vitro evaluation of BP-1-102 analogs with modified hydrophobic fragments for STAT3 inhibition. J Enzyme Inhib Med Chem 2021 36(1):410-424.
5. Truxova I, Kasikova L, Salek C, Hensler M, Lysak D, Holicek P, Bilkova P, Holubova M, Chen X, Mikyskova R, Reinis M, Kovar M, Tomalova B, Kline JP, Galluzzi L, Spisek R, Fucikova J*. Calreticulin exposure on malignant blasts correlates with improved natural killer cell-mediated cytotoxicity in acute myeloid leukemia patients. Haematologica. 2020 105(7):1868-1878.

LABORATORY OF

VIRAL AND CELLULAR GENETICS

Retroviruses, endogenous retroviruses, receptors for retrovirus entry, restriction factors, antivirus innate immunity, gene editing in chicken, syncytins, epigenetic suppression of retroviruses, chicken genetics and genomics

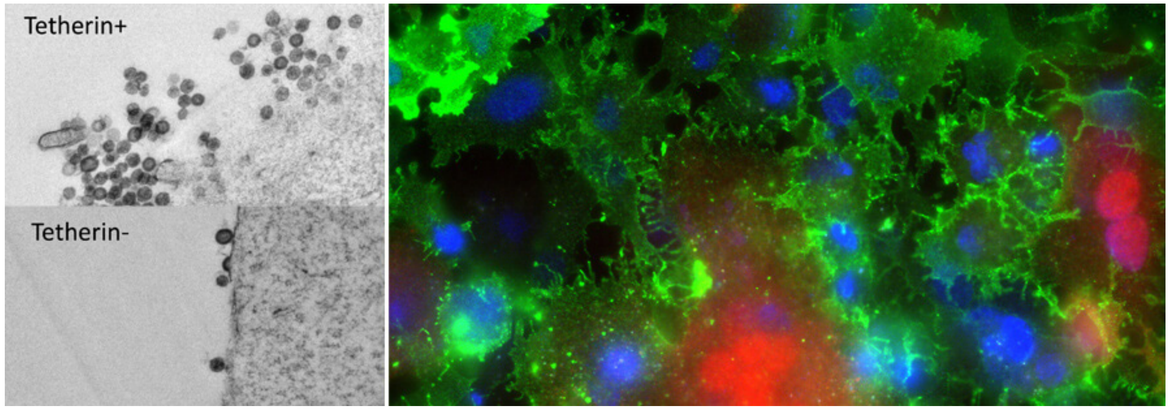
Jiří Hejnar

In the picture: 1. Reinišová Markéta | 2. Hron Tomáš | 3. Trejbalová Kateřina | 4. Matoušková Magda | 5. Pečenka Vladimír | 6. Slavková Martina | 7. Kučerová Dana | 8. Pecnová Ľubomíra | 9. Gáliková Eliška | 10. Elleder Daniel | 11. Bendová Michaela | 12. Stepanets Volodymyr | 13. Ungrová Lenka | 14. Hejnar Jiří | 15. Trávníček Martin | 16. Štafl Kryštof | 17. Prost Salomé | 18. Miklík Dalibor | 19. Karafiát Vít | 20. Kaňka Jakub | 21. Geryk Josef

Replication of retroviruses in host cells is a result of the interplay between the virus and multiple cellular factors, either the virus dependence factors necessary for subsequent steps of the replication cycle or restriction factors that block virus replication and contribute to the innate antiviral immunity. The major focus of our group are receptors for retroviruses that specifically attach the virus and assist in virus entry. Avian leukosis virus (ALV), an important pathogen in the domestic chicken, diversified into several subgroups differing in their receptor usage. This creates an opportunity to study the virus-host coevolution and accommodation of the virus to a new host. As a practical outcome of this research, we pioneer the techniques of CRISPR/Cas9 genome editing of the chicken (together with partners from commercial sphere) and manipulate the genes for ALV receptors with the aim to obtain virus-resistant chicken lines (1, 2). Physiological functions of ALV receptors are another domain of our interest (3).

Working with avian retroviruses, we also need a strong background in the genetics and genomics of birds, particularly the chicken. Our analyses of the GC-rich parts of the chicken genome led to the discovery of several genes formerly considered to have been lost in the lineage of galliform birds (TNF-a, BST-2, PTX3, EPO, EPOR, etc). In combination with CRISPR/Cas9 gene editing either in cell lines or in vivo, we are now able to test the biological activities of these genes and compare them with functions in other vertebrates. For example, chicken BST-2/Tetherin exerts antiviral activity against ALV (4, Fig. 1) and potentially against other enveloped viruses (avian flu, avian coronaviruses, etc.). Factors of innate immunity will be of particular interest in the future.

We are also interested in endogenous retroviruses in the genome of vertebrates, which are the signs of ancient infections. Previously, we identified endogenous lentiviruses and deltaretroviruses in various mammalian lineages – the oldest HIV- and HTLV-related molecular fossils. Endogenous retroviruses adopt unexpected functions like syncytin-1, which is inevitable for cell-to-cell fusion in human placenta (Fig. 2). We focus on the interaction of syncytin-1 with its specific receptor (hASCT2 amino acid transporter) and cell-to-cell fusion (5), which occurs physiologically in the placenta and aberrantly in germline tumours.



Left: Retrovirus particles are retained in clusters on the cell surface in the presence of tetherin (top), whereas individual particles bud and are released from the host cell (bottom). Right: Chicken cells artificially fused into multinuclear syncythia by ectopic expression of human ASCT2 and syncytin-1. Green colour, ASCT2 fused with AcGFP; red colour, cells co-expressing syncytin-1 and dsRed; blue colour, cell nuclei stained with DAPI.

Selected publications:

1. Koslová A, Trefil P, Mucksová J, Reinišová M, Plachý J, Kalina J, Kučerová D, Geryk J, Krchlíková V, Lejčková B, Hejnar J*: Precise CRISPR/Cas9 editing of the NHE1 gene renders chickens resistant to the J subgroup of avian leukosis virus. Proc Natl Acad Sci USA 2020 117(4):2108-2112.
2. Koslová A, Trefil P, Mucksová J, Krchlíková V, Plachý J, Krijt J, Reinišová M, Kučerová D, Geryk J, Kalina J, Šenigl F, Elleder D, Kožich V, Hejnar J*: Knock-out of retrovirus receptor gene tva in the chicken confers resistance to avian leukosis virus subgroups A and K and affects cobalamin (vitamin B12)-dependent level of methylmalonic acid. Viruses 2021 13(12):2504.
3. Krchlíková V, Mikešová J, Geryk J, Bařinka C, Nexo E, Fedosov SN, Kosla J, Kučerová D, Reinišová M, Hejnar J, Elleder D*: The avian retroviral receptor Tva mediates the uptake of transcobalamin bound vitamin B12 (cobalamin). J Virol 2021 95(8):e02136-20.
4. Krchlíková V, Fábryová H, Hron T, Young JM, Koslová A, Hejnar J, Strebel K, Elleder D*: Antiviral activity and adaptive evolution of avian tetherins. J Virol 2020 94(12):e00416-20.
5. Štafl K, Trávníček M, Kučerová D, Pecnová Ľ, Krchlíková V, Gáliková E, Stepanets V, Hejnar J, Trejbalová K*: Heterologous avian system for quantitative analysis of Syncytin-1 interaction with ASCT2 receptor. Retrovirology 2021 18(1):15.

LABORATORY OF

IMMUNOBIOLOGY

Central and peripheral immune tolerance, extrathymic function of Aire, TCR signalling, TLR signalling, embryonic haematopoiesis

Dominik Filipp

In the picture: 1. Faltýnková Petra | 2. Petrusová Jana | 3. Sakanwi Joy | 4. Jančovičová Kristýna | 5. Tahtahová Valérie | 6. Petrezselyová Silvia | 7. Čepková Adéla | 8. Machač David | 9. Sýkora Vojtěch | 10. Ballek Ondřej | 11. Puskeiler Matůš | 12. Březina Jiří | 13. Filipp Dominik

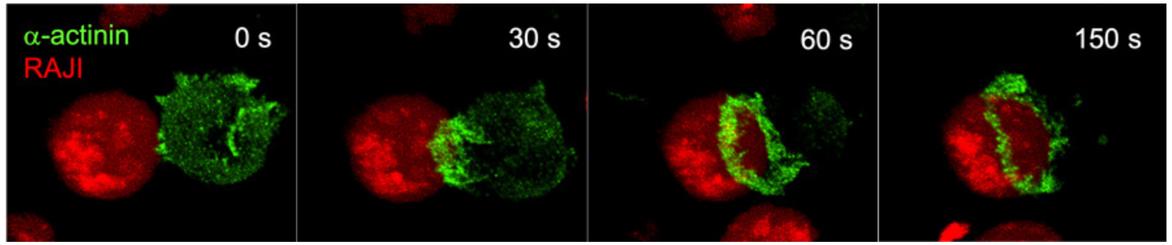
Our research mainly concerns:

1. the mechanisms guiding the process of immune central and peripheral T-cell tolerance and autoimmunity;
2. initiation of T-cell activation;
3. embryonic haematopoiesis, and
4. the role of Toll-like receptors in these processes.

In the last decade, we dedicated our effort to improve the impact of our research activity by implementing the following four strategies:

1. in general, we strictly set up our priorities and select those areas of research that are highly novel, promising, competitive, and yet close to our expertise and interest;
2. particularly, we focused on a deeper and more comprehensive understanding of the cellular, molecular and signalling aspects of the mechanisms of central and peripheral tolerance, embryonic homeostasis and T-cell signalling. Towards this end, we utilized the strategy of generating several knock-out and knock-in transgenic mouse strains, which allowed us to observe the biological correlates and consequences of these mechanisms under perturbed and unperturbed conditions;
3. we heavily invested in the acquisition of a battery of commercially or academically available transgenic mouse models and created an experimental panel of mutated strains, which allowed us to accelerate the rate of discovery; and
4. we further deepen our ties with collaborating laboratories abroad and locally, organize regular meetings and exchanges of ideas, materials and reagents.

In this context, we take pride in our highly motivated, smart and hard-working students and young scientists and the fact that all lab members are actively involved in shaping the research via discussions at lab meetings, preparing talks for conferences and students' seminars.



Kinetics of immunological synapse formation between RAJI B-cell (red) and Jurkat T-cell expressing the cytoskeletal component a-actinin-1 (green). Microscopy was performed using an Andor Dragonfly Spinning disc confocal microsope.

Selected publications:

1. Vobořil M, Brabec T, Dobeš J, Šplíchalová I, Březina J, Čepková A, Dobešová M, Aidarova M, Kubovčiak J, Tsyklauri O, Štěpánek O, Beneš V, Sedláček R, Klein L, Kolář M, and Filipp D. Toll-like receptor signaling in thymic epithelium controls monocyte-derived dendritic cell recruitment and Treg generation. 2020. Nature Communication, 11:2361, 1-16.
2. Vobořil M, Březina J, Brabec T, Dobeš J, Ballek O, Dobešová M, Manning J, Blumberg RS, Filipp D. A model of preferential pairing between epithelial and dendritic cells in thymic antigen transfer. 2022. eLIFE, 11:e71578, 1-18.
3. Dobes J, Binyamin A, Oftedal B, Goldfarb Y, Kadouri N, Gropper Y, Giladi T, Filipp D, Husebye ES, Abramson J. Aire-expressing ILC3 like cells are essential for induction of Candida-specific Th17 response. 2022. Nature Immunology, 23(7):1098-1108.
4. Březina J, Vobořil M and Filipp D. Mechanisms of direct and indirect presentation of self-antigens in the thymus. 2022, Frontiers in Immunology, 13:926625, 1-13.
5. Petrusová J, Manning J, Kubovčiak J, Kolář M and Filipp D. Two complementary approaches for efficient isolation of Sertoli cells for transcriptomic analysis. Front. Cell Dev. Biol. 10:972017, 1-12.
6. Petrusová J, Manning J and Filipp D. AIRE in male fertility: a new hypothesis. 2022, Cells, 11, 3168, 1-11.

LABORATORY OF

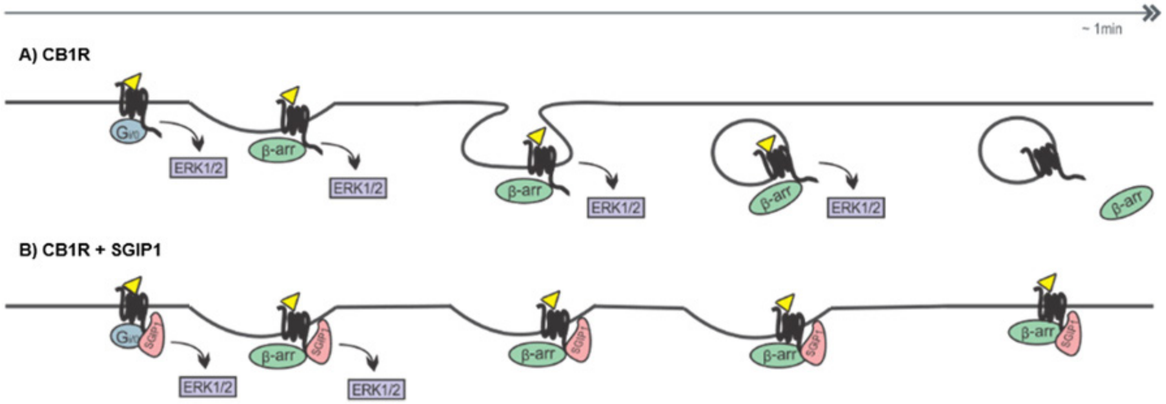
MOLECULAR PHARMACOLOGY

Endocannabinoids, cannabinoid receptor, receptors coupled to G-proteins

Jaroslav Blahoš

In the picture: 1. Gazdarica Matej | 2. Blahoš Jaroslav | 3. Cheveleva Irina | 4. Durydivka Oleh

We detected the Src homology 3-domain growth factor receptor-bound 2-like (endophilin) interacting protein 1 (SGIP1) as a novel cannabinoid receptor 1 (CB1R) interacting partner. SGIP1 is functionally linked to clathrin-mediated endocytosis, and its overexpression in the hypothalamus leads to an energy regulation imbalance resulting in obesity in rodents. We reported that SGIP1 prevents endocytosis of activated CB1R and that it alters signalling via CB1R in a biased manner. CB1R – beta 2 arrestin-associated signalling is profoundly changed, most likely as a consequence of prevention of the receptor's internalization, an inhibition mediated by SGIP1. To study the role of SGIP1 in vivo, we developed SGIP1 knockout mice to explore their phenotype. In a recent manuscript, we report alterations in the emotionality of SGIP1 knockout mice based on open field, elevated plus maze, and light/dark box tests. We discovered that mouse coping with despair in an inescapable situation is enhanced by SGIP1 deletion. In the tail immersion test, the antinociceptive effects of CB1R agonists were significantly enhanced in the SGIP1 knockout mice. In evaluating responses to D9-tetrahydrocannabinol in cannabinoid tetrad tests, interesting differences were found compared to wild-type mice, including modification of responses in models of acute, and chronic pain.



Proposed model of SGIP1 effect on CB1R internalization and signalling. A) Transfected cells without SGIP1. Upon CB1R agonist-induced signalling through Gi/o, the receptor associates with b-arrestin and is readily internalized, which allows massive ERK1/2 activation. B) In cells expressing SGIP1, internalization is prevented at early stages. Therefore, G-protein signalling is unaltered. However, the extent of CB1R signalling via the ERK1/2 pathway is decreased, as SGIP1 competes with FCHo1/2 proteins required for the initial stages of clathrin-coated pit formation. Thus, the internalization of CB1R is abrupt at the early stage, when the pits are in the initial phases of growth, or even before this event. SGIP1 prevents ERK1/2 signalling of the receptor-b-arrestin complex that would occur during further steps of internalization. Conversely, b-arrestin association with CB1R is enhanced, as their dissociation, which normally occurs in internalized endocytic compartments, does not occur.

Selected publications:

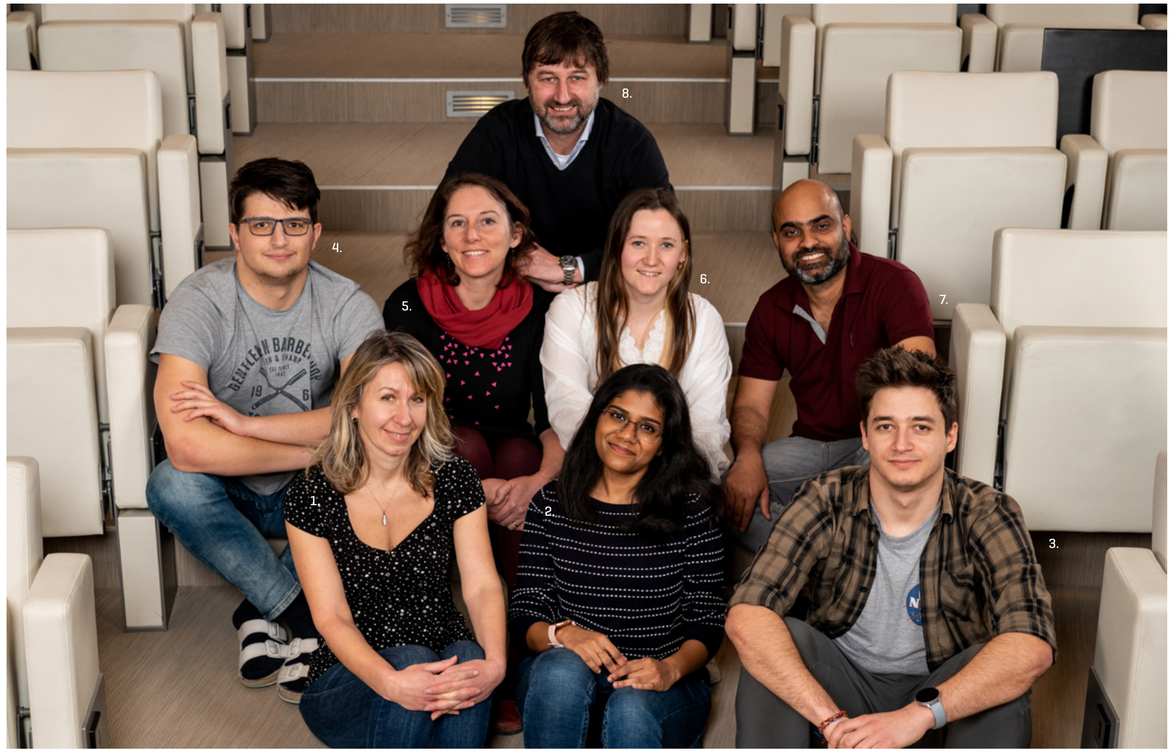
1. SGIP1 alters internalization and modulates signaling of activated cannabinoid receptor 1 in a biased manner. Hájková A, Techlovská Š, Dvořáková M, Chambers JN, Kumpošt J, Hubálková P, Prezeau L, Blahos J.*. Neuropharmacology. 2016 Aug;107:201-214. doi: 10.1016/j.neuropharm.2016.03.008. Epub 2016 Mar 9.
2. SGIP1 is involved in regulation of emotionality, mood, and nociception and modulates in vivo signalling of cannabinoid CB1 receptors. Dvorakova M, Kubik-Zahorodna A, Straiker A, Sedlacek R, Hajkova A, Mackie K, Blahos J.*. Br J Pharmacol. 2021 Apr;178(7):1588-1604.
3. SGIP1 modulates kinetics and interactions of the cannabinoid receptor 1 and G protein-coupled receptor kinase 3 signalosome. Gazdarica M, Noda J, Durydivka O, Novosadova V, Mackie K, Pin JP, Prezeau L, Blahos J.*. J Neurochem. 2022 Mar;160(6):625-642.

LABORATORY OF

RNA BIOLOGY

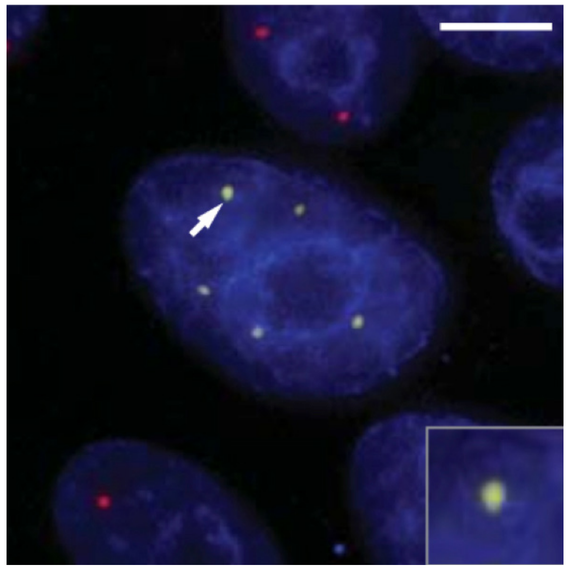
RNA splicing, nuclear structures, spliceosome, retinitis pigmentosa

David Staněk



In the picture: 1. Machatová Křížová Jana | 2. Banik Poulami | 3. Radivojević Nenad | 4. Karásek Filip | 5. Cvačková Zuzana | 6. Hana Petržílková | 7. Thakur Kumar Prasoon | 8. Staněk David | Missing in photo: Felix Zimmann, Yelyzaveta Pakhomova.

Our DNA contains the information for the synthesis of all our proteins. However, this information in human DNA is fragmented and our genes contain long, seemingly "useless" sequences that need to be removed in a process called RNA splicing. The „useless" RNA sequences are removed by a large, sophisticated and dynamic molecular machine called the spliceosome. The spliceosome is one of the most complex particles in our cells, composed of several non-coding RNAs and ~150 accessory proteins. Our long-term goal is to determine how the spliceosome assembles at the right time and place inside the cell. We are investigating how nuclear architecture contributes to the correct spliceosome formation, and studying the molecular principles of the control mechanism that distinguishes correctly assembled spliceosome particles from the defective ones. We recently identified a new protein that assists in assembly and recycling of U5 snRNP - one of the essential building block of the spliceosome. We further investigated maturation process of snRNAs - crucial components of the spliceosome. We discovered a molecular mechanism that cells use to identify and eliminate defective snRNA transcripts. Finally, we seek to determine why mutations in several ubiquitously expressed spliceosomal components cause retinitis pigmentosa, a human genetic disease characterized by photoreceptor cell degeneration. We mapped how a retinitis pigmentosa-linked mutation in one of the key spliceosomal RNA helicases affects its function and RNA splicing.



In vitro transcribed snRNA labelled by Alexa488-UTP (green) was microinjected into HeLa cells snRNA localizes in Cajal bodies yellow/red (arrow). DNA was labelled by DAPI (blue). Cajal body was magnified two times in inset. Bar represents 5μm.

Selected publications:

1. Basello A.D., Matera A.G.& Staněk D.* (2022) A point mutation in human coilin prevents Cajal body formation. Journal of Cell Science. 135 (8): jcs259587.
2. Cihlářová Z., Kubovčiak J., Sobol M., Krejčíková K., Sachová J., Kolář M, Staněk D., Bařinka C., Yoon G., Caldecott K.W. & Hanzlíková H.* (2022) BRAT1 Links Integrator and Defective RNA Processing with Neurodegeneration. Nature Communications 13(1):5026
3. Obuca M., Cvačková Z., Kubovčiak J., Kolář M. & Staněk D.* (2022) Retinitis pigmentosa-linked mutation in DHX38 modulates its splicing aktivity. PLoS ONE. 17(4):e0265742
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5. Roithová A., Feketová Z., Vaňáčová Š. & Staněk D.* (2020) DIS3L2 and LSm proteins are involved in the surveillance of Sm ring-deficient snRNAs. Nucleic Acids Research. 48(11):6184-6197

LABORATORY OF

TRANSGENIC MODELS OF DISEASES

Genome editing, mouse models of human diseases, craniofacial and skeleton development, proteases, UB-ligases

Radislav Sedláček

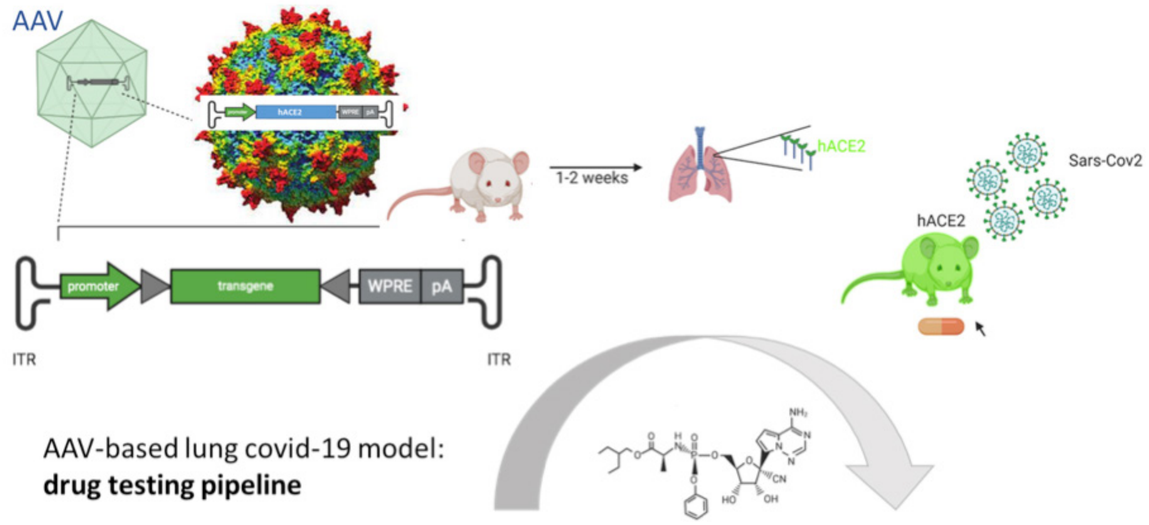
In the picture: 1. Procházka Jan | 2. Aranaz Novaliches Goretti | 3. Šímová Michaela | 4. Ogan Betul Melike | 5. Sedláček Radislav | 6. Kašpar Petr | 7. Turečková Jolana | 8. Procházková Michaela | 9. Iatsiuk Veronika | 10. Raishbrook Miles

The Laboratory of Transgenic Models of Diseases combines utilization of cutting-edge genome-editing technologies and in-depth phenotyping analysis of an animal model to uncover novel genetic mechanisms for selected human diseases. We use the CRISPR/Cas9-based technique and various molecular-biology approaches to generate novel genetic mouse models or study the function of a particular gene in cells, ex vivo, or to deliver targeting or therapeutic vectors into the organism.

Our group has several areas of interest; all of them are connected via mouse models helping us to decipher either new biological roles that have not been described yet, or to understand pathologic gene variants in the development of a human disease.

Thus, we study the role of cullin-RING ubiquitin ligases involved in GIT homeostasis and pathological processes, since the cullin family has been largely associated with different types of cancer in GIT and thus represents a promising pharmacological target (Btbd3, Rnf121, Rnf186, Cul4a, Ddb1, Cul3, and others). In the field of proteases, we focus on studies of several metalloproteinases (e.g., Trabd2b), and especially on the functional redundancy of kallikreins and their inhibitors. If they are dysregulated (e.g., in the rare disease of Netherton syndrome), they have deleterious consequences in the disease development. In the field of craniofacial, skeleton and teeth development, we focus on the molecular mechanism driving the fascinating complex process of craniofacial and skeleton development, unveiling molecular regulation of epithelial morphogenesis and involvement of ubiquitin-dependent proteolytic pathways in the regulation of morphogenetic signalling cascades. One of the most striking features of craniofacial area is development of mineralized tissues such as teeth and bones. Here, we study the function of extracellular proteins, including ameloblastin, in the regulation of mineralization processes in tooth enamel formation and bone homeostasis process, revealing, for instance, how the FAM46A loss-of-function mutation was found in patients with osteogenesis imperfecta.

In 2020, in response to the pandemic situation based on Covid-19, we have developed several mouse models (Nature, 2021 May;593(7859):424-428) to study the biological aspects of SARS-Cov-2 infection in the mouse as a model organism and to develop therapies to this deleterious infection.



Selected publications:

1. Bispecific IgG neutralizes SARS-CoV-2 variants and prevents escape in mice. De Gasparo R, Pedotti M, Simonelli L, Nickl P, Muecksch F, Cassaniti I, Percivalle E, Lorenzi JCC, Mazzola F, Magri D, Michalcikova T, Haviernik J, Honig V, Mrazkova B, Polakova N, Fortova A, Tureckova J, Iatsiuk V, Di Girolamo S, Palus M, Zudova D, Bednar P, Bukova I, Bianchini F, Mehn D, Nencka R, Strakova P, Pavlis O, Rozman J, Gioria S, Sammartino JC, Giardina F, Gaiarsa S, Pan-Hammarström Q, Barnes CO, Bjorkman PJ, Calzolai L, Piralla A, Baldanti F, Nussenzweig MC, Bieniasz PD, Hatziioannou T, Prochazka J, Sedlacek R, Robbiani DF, Ruzek D, Varani L. Nature. 2021 May;593(7859):424-428. doi: 10.1038/s41586-021-03461-y. Epub 2021 Mar 25. PMID: 33767445

LABORATORY OF

CELL AND DEVELOPMENTAL BIOLOGY

Stem cells, signalling pathways, gastrointestinal tract, cancer, haematological disorders

Vladimír Kořínek

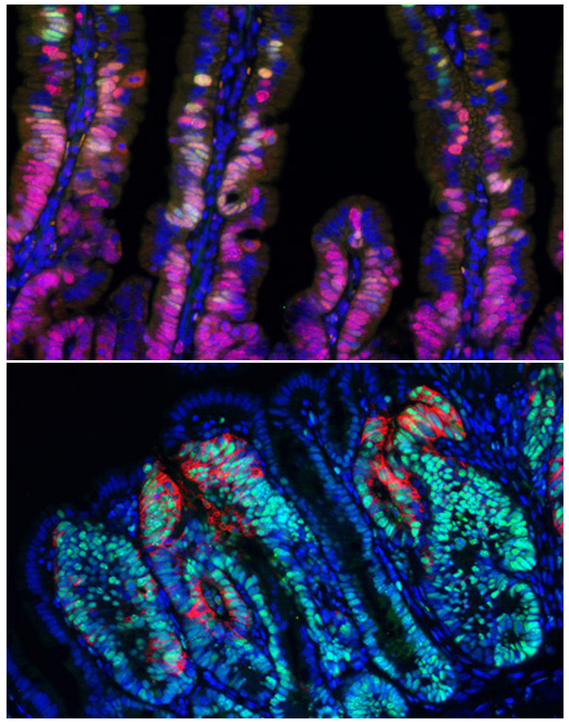
In the picture: 1. Kořínek Vladimír | 2. Kříž Vítězslav | 3. Danačíková Šárka | 4. Onhajzer Jakub | 5. Hrčkulák Dušan | 6. Šloncová Eva | 7. Galušková Kateřina | 8. Zimolová Veronika

The tissues of the adult organism contain a population of tissue-specific stem cells that form the cellular basis for the homeostatic maintenance of the adult tissue. Our aim is to elucidate the molecular mechanisms that influence the fate of normal and transformed adult stem cells in the intestine and the haematopoietic system.

Since the fate of intestinal stem cells is determined by the Wnt signalling pathway, one of our goals is to find and characterise genes that are regulated by this pathway. The first oncogenic mutation is thought to confer a selective advantage to future cancer cells that proliferate and form the first neoplastic lesion. Interestingly, our results suggest that the transformed cell activates a specific transcriptional programme depending on its position in the gut to ensure its long-term survival in the tissue. Importantly, only a cell that remains in the body for a long time can accumulate additional changes that drive tumour growth and progression. The identity of somatic stem cells is determined by a specific microenvironment called the stem cell niche, which allows tight control of tissue homeostasis. Recently, we discovered that subepithelial mesenchymal cells form the intestinal stem cell niche by secreting Wnt ligands that promote stem cell renewal. We further characterise specific features of niche cells.

Our research interests also include the molecular basis of haematological diseases, especially disorders related to red blood cell production and renewal. Myeloproliferative neoplasms (MPN) represent a group of diseases that arise from genetic defects in haematopoietic stem cells. Our aim is to identify the genetic predispositions for MPN and define their impact on different cellular backgrounds. We have shown that germline (or acquired) mutations in the gene encoding Janus kinase 2 (JAK2) amplify the oncogenic JAK2/STAT signalling pathway and cause a specific clinical course of the disease in MPN patients.

We use genetically modified (transgenic, gene knockout or knock-in) mice as the main experimental tool. Besides in vivo models, we also use intestinal organoid cultures obtained from healthy or tumour tissue. To investigate the genetic basis of haematological malignancies, we perform next-generation sequencing of genomic DNA isolated from human samples.



Top: Fluorescence microphotography documenting the heterogeneity of transformed cells in the early stages of tumour formation. Localization of Msx1 (green fluorescence signal) and PCNA (red fluorescence signal) proteins in a mouse model of small bowel cancer 4 days after the first oncogenic mutation. Samples were counterstained with DAPI (blue fluorescent nuclear signal). Actively proliferating cells are PCNA positive (note that the purple staining is the result of coalescence of blue and red fluorescent signal). These cells are mainly found in epithelial invaginations, the crypts. In contrast, Msx1 (mainly) labels transformed cells in the villi of the small intestine; some of these cells express Msx1 and PCNA together (yellow fluorescence). Bottom: Fluorescence micrograph of epithelial cells stained for the proliferation marker PCNA (green fluorescence signal) and cells positive for Trop2 protein (red fluorescence signal) in small intestinal tumors arising in an experimental mouse 6 weeks after inactivation of the tumor suppressor Apc; the sample was contrast stained with DAPI (blue fluorescence signal in the nucleus).

Selected publications:

1. Švec J, Šťastná M, Janečková L, Hrčkulák D, Vojtěchová M, Onhajzer J, Kříž V, Galušková K, Šloncová E, Kubovčiak J, Pfeiferová L, Hrudka J, Matěj R, Waldauf P, Havlůj L, Kolář M, Kořínek V.*: TROP2 Represents a Negative Prognostic Factor in Colorectal Adenocarcinoma and Its Expression Is Associated with Features of Epithelial-Mesenchymal Transition and Invasiveness. Cancers (Basel) 2022 14(17).
2. Olbertová K, Hrčkulák D, Kříž V, Jesionek W, Kubovčiak J, Ešner M, Kořínek V, Buchtová M: Role of LGR5-positive mesenchymal cells in craniofacial development. Front Cell Dev Biol 2022 10: 810527
3. Degirmenci B, Dincer C, Demirel HC, Berkova L, Moor AE, Kahraman A, Hausmann G, Aguet M, Tuncbag N, Valenta T*, Basler K*: Epithelial Wnt secretion drives the progression of inflammation-induced colon carcinoma in murine model. iScience 2021 24(12): 103369.
4. Kriska J, Janeckova L, Kirdajova D, Honsa P, Knotek T, Dzamba D, Kolenicova D, Butenko O, Vojtechova M, Capek M, Kozmik Z, Taketo MM, Korinek V, Anderova M: Wnt/β-Catenin Signaling Promotes Differentiation of Ischemia-Activated Adult Neural Stem/Progenitor Cells to Neuronal Precursors. Front Neurosci 2021 15: 628983.
5. Danek P, Kardosova M, Janeckova L, Karkoulia E, Vanickova K, Fabisik M, Lozano Asencio C, Benoukraf T, Tirado-Magallanes R, Zhou Q, Burocziova M, Rahmatova S, Pytlik R, Brdicka T, Tenen DG, Korinek V, Alberich Jorda M: β-catenin-TCF/LEF signaling promotes steady-state and emergency granulopoiesis via G-CSF receptor upregulation. Blood 2020: 136(22):2574-258.

LABORATORY OF

MOUSE MOLECULAR GENETICS

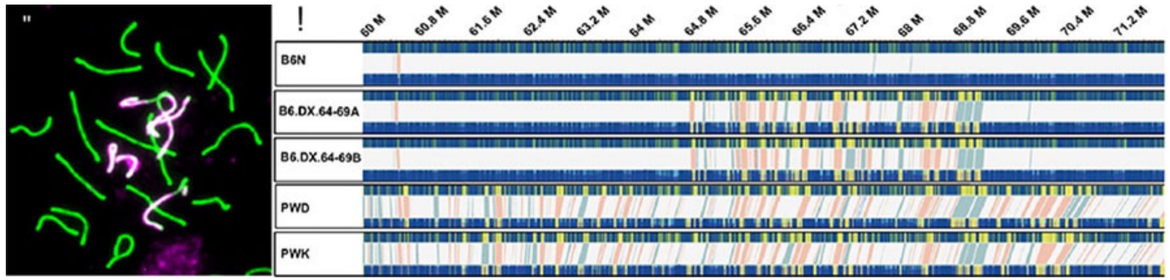
Mouse subspecies, speciation, *Prdm9*, meiotic recombination and chromosome pairing, synaptonemal complex, X-chromosome inactivation

Jiří Forejt

In the picture: 1. Dobišová Zuzana | 2. Jansa Petr | 3. Fusek Karel | 4. Forejt Jiří | 5. Tanieli Giordano

Hybrid sterility is defined as a situation when two species, each of which is fertile, produce a hybrid progeny, which is sterile. Hybrid sterility thus disrupts free exchange of genes between populations, an essential prerequisite for origin of new species. We identified the first and up to now the sole "speciation" gene in mammals, which controls hybrid sterility between two mouse subspecies. This gene, PR domain-containing 9 *(Prdm9)*, determines the sites of genetic recombination in the genome of mice and most other mammals. In sterile hybrids, PRDM9 allelic incompatibility causes disorders in DNA repair and in meiotic chromosome pairing, resulting in male infertility. Another, still undiscovered major hybrid sterility gene in mice is located within the Hstx2 locus on Chromosome X and modulates the effect of *Prdm9*.

We are currently trying to identify the X-linked hybrid sterility gene and to understand the molecular mechanism of its interaction with *Prdm9* and other minor hybrid sterility genes. We use the combination of high-resolution whole-genome genetic mapping and RNA-seq for molecular profiling of isolated populations of spermatogenic cells. By combining immunofluorescence microscopy techniques and fluorescence in-situ hybridization, we study meiotic chromosome pairing and synapsis and male-specific X-chromosome transcriptional inactivation, which are the processes affected in sterile male hybrids. Finally, we re-visit and re-define the chromosomal hybrid sterility by using the panel of inter-subspecific chromosome substitution strains in the *Prdm9*-controlled model of "synthetic hybrid sterility."



Left: Immunofluorescence microscopy of nuclear spread of chromosomes from primary spermatocyte at pachytene stage from a sterile hybrid male. Synaptonemal complexes of individual chromosome pairs are visualized by immunostaining of SYCP3 protein (green). Asynapsed chromosomes are decorated by anti-HORMAD2 antibody (orange). From: Forejt J. Can a non-coding genomic sequence provoke infertility of interspecific hybrids? Hommage to Theodosius Dobzhansky (Nature Portfolio Ecology & Evolution Community. May 31, 2018 (with permission). Right: Subspecies-specific structural variants (SVs) in the Hstx2 locus and in flanking regions revealed by genome optical mapping (Bionano Genomics). Each box contains a comparative analysis of a de-novo optical map (bottom), and the mm10 genome assembly in-silico reference B6 map (top) representing domesticus and musculus mouse subspecies (B6N, PWK, PWD) and a consomic strain (B6.DX.64-69A, B6.DX.64-69B). From: Lustyk D. et al. Genomic structucture of Hstx2 modifier of *Prdm9*-dependent hybrid male sterility (Genetics, Vol. 213, 1047–1063 November 2019).

Selected publications:

1. Valiskova B, Gregorova S, Lustyk D, Šimeček P, Jansa P, Forejt J*. Genic and chromosomal components of *Prdm9*-driven hybrid male sterility in mice (Mus musculus). Genetics. 2022 Aug 30;222(1):iyac116. doi: 10.1093/genetics/iyac116.
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3. Gergelits V, Parvanov E, Simecek P, Forejt J*. Chromosome-wide characterization of meiotic noncrossovers (gene conversions) in mouse hybrids. Genetics. 2021 Mar 3;217(1):1-14. doi: 10.1093/genetics/iyaa013.
4. Mukaj A, Piálek J, Fotopulosova V, Morgan AP, Odenthal-Hesse L, Parvanov ED*, Forejt J*. *Prdm9* Intersubspecific Interactions in Hybrid Male Sterility of House Mouse. Mol Biol Evol. 2020 Dec 16;37(12):3423-3438. doi: 10.1093/molbev/msaa167. PMID: 32642764; PMCID: PMC7743643

LABORATORY OF

SIGNAL TRANSDUCTION

Plasma membrane signalosomes, membrane receptor signalling, immunoreceptor regulators, bacterial cytolysins, mast cells

Petr Dráber

In the picture: 1. Dráberová Lubica | 2. Hálová Ivana | 3. Bugajev Viktor | 4. Utekal Pavol | 5. Mrkáček Michal | 6. Franková Daniela | 7. Dráber Petr | 8. Tůmová Magda

Our laboratory focuses on understanding the molecular mechanisms governing signal transduction from the plasma membrane receptors to the cytoplasm. We study mainly mast cells, potent immune modulators of the tissue microenvironment. In these cells, antigen-mediated aggregation of the immunoglobulin E receptor (FcεRI) results in degranulation and cytokines production. Increasing evidence suggests that an intricate network of inhibitory and activating receptors, together with lipids, governs the initiation of the mast cell's responsiveness to particular stimuli.

For our studies of plasma membrane signalling units, signalosomes, we use various techniques of molecular biology, immunology, and immunochemistry. Our principal approach lies in the production of cells or animals with increased or reduced expression of selected genes and comparing their properties with wild-type cells or wild-type animals.

Using mast cells from mice with ORMDL3 knockout (KO), we examined the role of ORMDL3 in mast cell activation. We found that the cells with ORMDL3 KO exhibited enhanced production of sphingolipids, as expected, because ORMDL3 is a negative regulator of the serine-palmitoyl transferase, a key enzyme in sphingolipid synthesis. Surprisingly, ORMDL-deficient cells also exhibited enhanced production of leukotrienes. We found that ORMDL3 physically interacts with 5-lipoxygenase, mediating enzyme conversion of arachidonic acid to leukotrienes. Several other experiments supported our hypothesis of physical and functional crosstalk between the leukotriene and sphingolipid metabolic pathways, leading to the production of lipid signalling mediators participating in signal transduction events and the development of several diseases.

An essential role of lipids was also demonstrated in our study examining the mechanism of the inhibitory effect of ursolic acid (UA) on the FcεRI-mediated activation of mast cells. UA has multiple biological activities. We found that UA rapidly reduced the mobility of plasma membrane components, including cholesterol and FcεRI. Based on our studies, we concluded that UA exerts its effects, at least in part, via lipid-centric plasma membrane perturbations, affecting the function of the FcεRI signalosome. These and other studies are directed to our long-term goal to contribute to the development of new, more potent, anti-allergic and anti-inflammatory drugs.

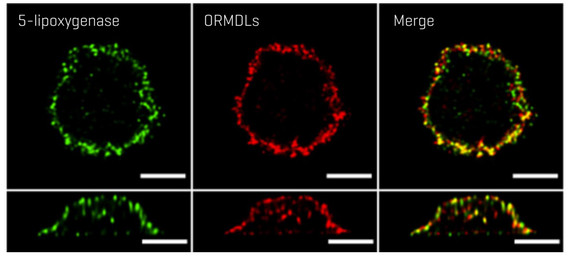

Figure 1. Data indicating that 5-lipoxygenase colocalizes with the ORMDLs on the endoplasmic reticulum membranes. Human mast cells HMC-1.1 were activated for 10 min with ionomycin and stained for 5-lipoxygenase and ORMDLs as described in Bugajev et al., J. Lipid Res., 2021. Co-localization is observed at both XY projections (top) and orthogonal projections (bottom).

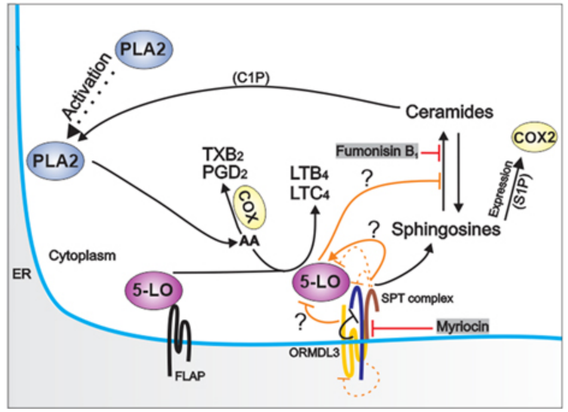

Figure 2. Scheme of the sphingolipid and eicosanoid metabolic pathway crosstalk. Interactions between the pathways seem to be mediated via metabolic mediators (ceramides, S1P, C1P) and physical interaction of the ORMDL3 with the SPT complex and 5-lipoxygenase. Details of the pathways are described in Bugajev et al., J. Lipid Res., 2021.

Selected publications:

1. Shaik GM, Draberova L, Cernohouzova S, Tumova M, Bugajev V, Draber P*. Pentacyclic triterpenoid ursolic acid interferes with mast cell activation via a lipid-centric mechanism affecting FcεRI signalosome functions. J Biol Chem 2022 298(11):102497.
2. Bugajev V*, Paulenda T, Utekal P, Mrkacek M, Halova I, Kuchar L, Kuda O, Vavrova P, Schuster B, Fuentes-Liso S, Potuckova L, Smrz D, Cernohouzova S, Draberova L, Bambouskova M, Draber P*. Crosstalk between ORMDL3, serine palmitoyltransferase, and 5-lipoxygenase in the sphingolipid and eicosanoid metabolic pathways. J Lipid Res 2021 62:100121.
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LABORATORY OF

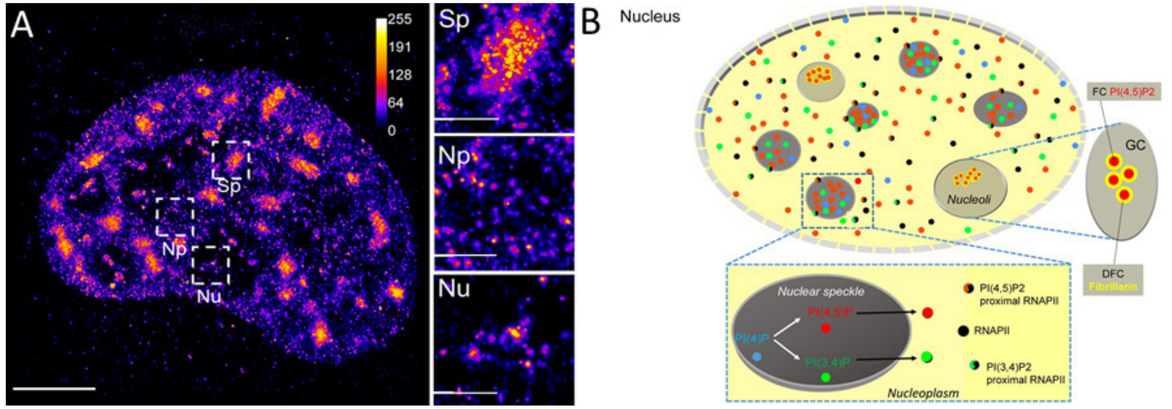
BIOLOGY OF THE CELL NUCLEUS

Nuclear lipids, phosphatidylinositol phosphates, nucleoskeleton, transcription, subnuclear compartments

Pavel Hozák

In the picture: 1. Antiga Ludovica | 2. Jelínková Iva | 3. Pišlová Lenkam | 4. Filimonenko Vláda | 5. Miladinović Ana | 6. Kříž Pavel | 7. Hoboth Peter | 8. Hozák Pavel | 9. Sztacho Martin

Functional nuclear sub-compartments linked with the crucial cellular processes, such as gene expression, contain phosphoinositides in the form of small foci (Fig. 1). Nevertheless, current models of gene expression largely omit the roles of nuclear lipids and amongst them nuclear phosphatidylinositol phosphates. We aim to fill this gap, and to this end, we use a combination of advanced light and electron microscopy (Fig. 2) together with biochemistry and molecular biology. These approaches allow us to gain insight into the role of nuclear phosphatidylinositol phosphates in the regulation of gene expression and push the boundaries of the field. We identified a novel type of nuclear structures – nuclear lipid islets – that contain phospholipids, proteins, and nucleic acids and serve as platforms for efficient transcription (Sobol et al., 2018). Our efforts continue to systematically unravel the phospholipid identity of the gene expression compartments (Hoboth et al., 2021), and we are testing the idea that PIs and their interactors regulate, at the molecular level, subsequent stages of RNAPII transcription (Sztacho et al., 2021). Moreover, our group has identified nuclear myosin I as a novel transcription factor (Philimonenko et al., 2004) and also contributed to understanding the role of nuclear actin and actin-related proteins in the establishment of functional nuclear architecture and regulation of gene expression (Balaban et al., 2021). Our research centred on the role of nuclear lipids in the formation of lipo-ribonucleoprotein transcription hubs is important for better understanding the role of functional nuclear architecture, nucleoskeleton, and nuclear lipids in the gene expression in health and disease.



Left: Fluorescent super-resolution imaging of nuclear phosphatidylinositol 4,5-bisphosphate. Selected regions show a specific pattern of PIP2 associated with nuclear speckles (Sp), nucleoplasm (Np) and nucleolus (Nu). © Hoboth et al. Int. J. Mol. Sci. 2021. Right: Detailed map of the sub-nuclear localization of PI(4,5)P2, PI(3,4)P2 and PI(4)P within nuclear speckles, in the proximity of a subset of RNAPII in the nucleoplasm and within the nucleolus. © Hoboth et al. BBA – Mol. Cell. Biol. Lipids 2021.

Selected publications:

1. Petrusová J, Havalda R, Flachs P, Venit T, Darášová A, Hůlková L, Sztacho M, Hozák P*: Focal Adhesion Protein Vinculin Is Required for Proper Meiotic Progression during Mouse Spermatogenesis. Cells 2022 11(13).
2. Balaban C, Sztacho M, Blažíková M, Hozák P*: The F-Actin-Binding MPRIP Forms Phase-Separated Condensates and Associates with PI(4,5)P2 and Active RNA Polymerase II in the Cell Nucleus. Cells 2021 10(4).
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LABORATORY OF

BIOLOGY OF CYTOSKELETON

Microtubules, tubulin isotypes, γ-tubulin complexes, regulation of microtubule nucleation, signal transduction

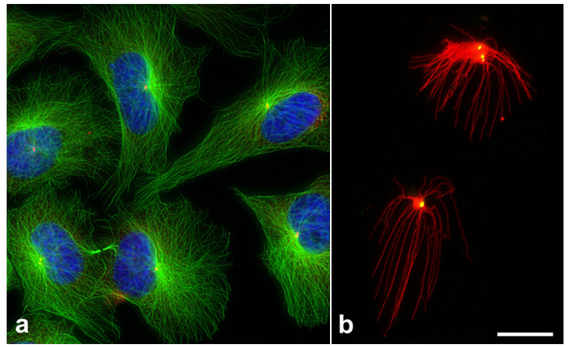
Pavel Dráber

In the picture: 1. Dráberová Eduarda | 2. Dráber Pavel | 3. Sulimenko Tetyana | 4. Vosecká Věra | 5. MIchová Irena | 6. Sulimenko Vadym

Microtubules are intracellular dynamic polymers made up of polymorphic αβ-tubulin heterodimers and a large number of microtubule-associated proteins. Microtubules are required for vital processes in eukaryotic cells, including cell division, maintenance of cell shape, intracellular transport, and signal transduction. The organization of microtubules in cells is controlled by microtubule organizing centres (MTOCs) as centrosomes. The key components of MTOCs are γ-tubulin complexes essential for microtubule nucleation. The regulatory mechanisms of γ-tubulin complex activation are just about to begin to be understood.

The long-term research programme of the laboratory includes study of the structure-function relationships of microtubule proteins and their interactions with other cytoskeletal elements in cells under normal and pathological conditions. In recent years, the research efforts have concentrated on elucidation of the molecular mechanisms governing microtubule nucleation and dynamics and the role of γ-tubulin in these processes. It has been shown that γ-tubulin is post-translationally modified and forms complexes with protein tyrosine kinases and phosphatases. An important role in the regulation of microtubule nucleation is played by the GIT/βPIX/PAK signalling complex. We have shown that the properties of γ-tubulin change during differentiation events and that γ-tubulin-2 could have a pro-survival function in neurons. The presence of γ-tubulin complex proteins was demonstrated in membrane-associated complexes and in the nuclei, where they modulate DNA damage G2/M checkpoint activation through tumour suppressor protein C53. We have also shown that C53 is important regulator of microtubule organization in cells under ER stress, and that microtubule dynamics in vitro and in cells could be modulated by nanosecond-pulsed electric fields.

Our current work focuses on deciphering the regulatory mechanisms of microtubule nucleation in activated mast cells and the role of signal transduction molecules in this event. We also study dysregulation of microtubule organization in brain cancer cells and the function of neuronal γ-tubulin-2 isotype. Finally, we define the new roles of actin-associated profilin in the regulation of centrosomal microtubule nucleation. To address these questions, we use techniques of molecular biology, biochemistry, and immunology, as well as a variety of microscopic techniques, including superresolution microscopy, live-cell imaging and quantification of microtubule nucleation and dynamics.



Microtubule nucleation from centrosomes in cells and in vitro.
(a) Human osteosarcoma cells U2OS stained for microtubules with antibody to β-tubulin (green) and for centrosomes with antibody to γ-tubulin (red). DNA in blue. (b) Centrosomes isolated from U2OS cells by sucrose gradient centrifugation were incubated with 1.2 mg/ml tubulin in the presence of 1 mM GTP for 20 min at 37°C. After fixation, centrosomes were centrifuged through a glycerol cushion onto a coverslip and immunostained for pericentrin to mark centrosomes (green) and for β-tubulin to mark microtubules (red). Scale bar for (a) and (b), 20 μm.

Selected publications:

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2. Klebanovych A, Vinopal S., Dráberová E., Sládková V., Sulimenko T., Sulimenko V., Vosecká V., Macůrek L., Legido A., Dráber P.*: C53 interacting with UFM1-protein ligase 1 regulates microtubule nucleation in response to ER stress. Cells 11: e555, 2022.
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5. Chafai D.E*., Vostárek F., Dráberová E., Havelka D., Arnaud-Cormos D., Leveque P., Janáček J., Kubínová L., Cifra M., Dráber P*: Microtubule cytoskeleton remodelling by nanosecond pulsed electric fields. Adv. Biosystems 4: e2000070, 2020.

LABORATORY OF

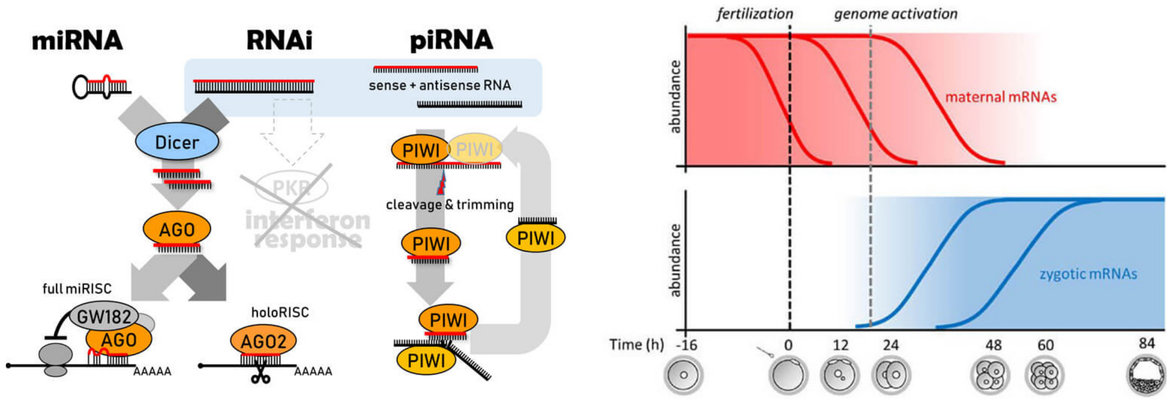
EPIGENETIC REGULATIONS

Oocyte-to-embryo transition, RNAi, miRNA, piRNA retrotransposon

Petr Svoboda

In the picture: 1. Florian Joseph Diego André | 2. Pasulka Josef | 3. Svoboda Petr | 4. Roos Kulmann Marcos Iuri | 5. Buccheri Valeria | 6. Kubíček Karel | 7. Ber Tobiáš | 8. Malík Radek

Despite the group's name, epigenetics is not its primary research area; the group owes its name to its early years, when ambitions to study epigenetic mechanisms were crushed by the lack of success to obtain funding for studying chromatin during mammalian oocyte-to-embryo transition. Nowadays, the group studies evolution of genes and their regulations, particularly post-transcriptional regulations, mainly in the context of the female germline in mice. However, there have been occasional detours into other research areas and model systems, such as oocytes of other mammals, zygotes, spermatogenesis, soma, and elsewhere. The major research theme during the recent years have been mammalian small RNA pathways (Fig. 1). The laboratory explored their functions and co-existence in mammalian germlines. We have found that these pathways dynamically evolved in mouse oocytes and we have been able to characterize some of the principles underlying function of small RNA pathways in oocytes and other cell types. We have explained how miRNAs loose activity in growing mammalian oocytes through dilution of the cytoplasmic content, which, at the same time, made oocytes permissive to canonical RNA interference (RNAi), which evolved there in rodents. The evolution has been accompanied by truncating Dicer, the key enzyme producing small RNAs. Mouse oocytes express high levels of a Dicer variant (Dicer^{0}), which is essential for development of meiotically and developmentally competent oocytes. It appears that the piRNA pathway has adapted along with RNAi, such that RNAi is dominant and essential in mouse females but not the piRNA, which is omissible there. In contrast, hamster oocytes require the piRNA pathway for developmental competence while RNAi pathway does not seem to be highly active. Lately, we have been trying to adapt RNA interference into an antiviral mechanism in vivo in mammals. We have been able to genetically engineer mice with somatic expression of Dicer^{0}, which have enhanced RNAi activity in soma, and with variable success we are analyzing antiviral effects of such a genetic modification. Additional research areas investigated in the lab recently include gene expression during mouse oocyte-to-embryo transition (Fig. 2) and long non-coding RNAs in mouse oocytes and zygotes.



Left: Overview of three mammalian small RNA pathways studied in the laboratory. Right: Oocyte-to-embryo-transition in mice | Oocyte-to-embryo transition has two major components: (I) maternal mRNA degradation, which erases oocyte's identity, and (II) zygotic genome activation, which drives establishment of zygote's new identity. In mice, the major zygotic genome activation takes place at the 2-cell stage. By that time, 75% of maternal mRNAs have been degraded.

Selected publications:

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3. Loubalova Z, Fulka H, Horvat F, Pasulka J, Malik R, Hirose M, Ogura A*, Svoboda P*. Formation of spermatogonia and fertile oocytes in golden hamsters requires piRNAs. Nat Cell Biol 2022 23(9):992-1001
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LABORATORY OF

TRANSCRIPTIONAL REGULATION

Development, evolution, gene regulation, transcription factors, eye

Zbyněk Kozmik

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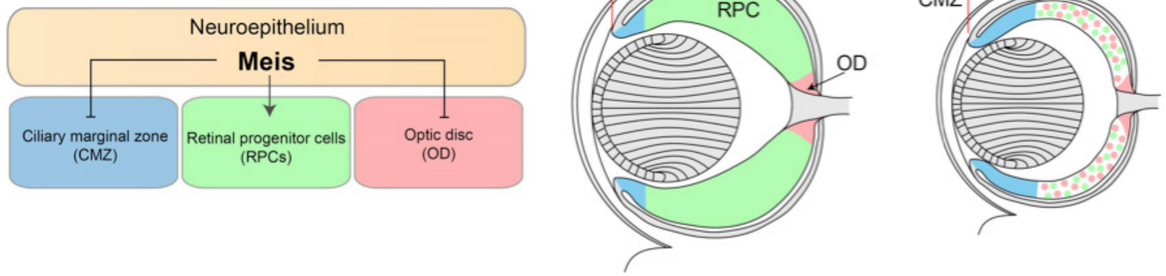
We investigate embryonic development using an integrative approach combining molecular biology, cell biology, developmental biology, genetics, biochemistry, and bioinformatics in order to get insight into the molecular mechanisms underlying the process of animal development and its tinkering during the course of evolution. We are especially interested in the role of transcription factors and signalling cascades integrated into complex gene regulatory networks. Several vertebrate and invertebrate model systems including mice, fish, amphioxus, annelids, and cnidaria are being used in the laboratory to study various aspects of animal development and evolution. The long-term interest of the group lies in the studies of vertebrate eye development, eye evolution, and body plan evolution.

The vertebrate eye development has been studied for a long time, but only in the last two decades the function of individual transcription factors began to be elucidated. Genetic manipulation in mice combined with interrogation of whole-genome occupancy of key transcription factors allows addressing the role which individual transcription factors play during embryonic development and how they interact with each other. Dissection of the regulatory networks will enhance our understanding of specific aspects of mammalian eye development and will lead to a more profound understanding of congenital eye defects in humans. We currently investigate the gene regulatory networks associated with transcription factors Pax6 and Meis during the retina, lens, and cornea development.

One of the most intriguing queries in developmental biology is how the specialized cell types, tissues, organs and the body plan evolved throughout the animal kingdom. We use invertebrate chordate amphioxus that has widely been used as a reference outgroup to infer ancestral versus novel features during vertebrate evolution. We identified Wnt/β-catenin signalling as an evolutionarily conserved determinant of chordate dorsal organizer, and provided insight into cell-type evolution in the chordate retina.



Meis homeobox genes control progenitor competence in the retina

Selected publications:

1. Sunny SS, Lachova J, Dupacova N, Kozmik Z*. Multiple roles of Pax6 in postnatal cornea development. Dev Biol 2022 491:1-12.
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LABORATORY OF

GENOMICS AND BIOINFORMATICS

Genomics, transcriptomics, bioinformatics, next-generation sequencing, single-cell approaches

Michal Kolář

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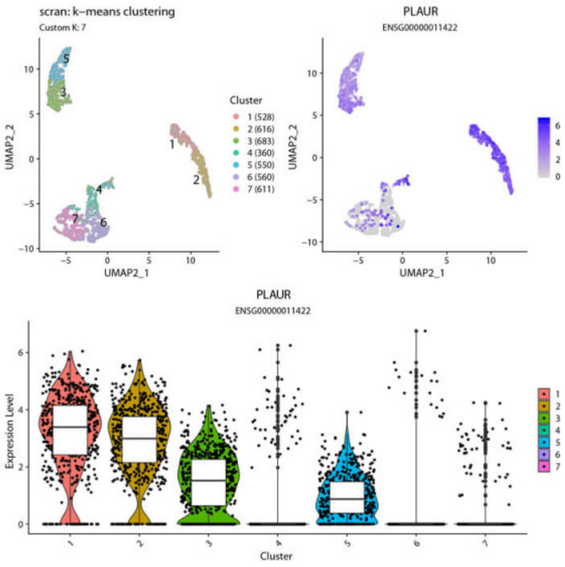
Activity of our laboratory is based on advanced applications of genomics, transcriptomics and bioinformatics, the most vigorously developing disciplines of contemporary life sciences.

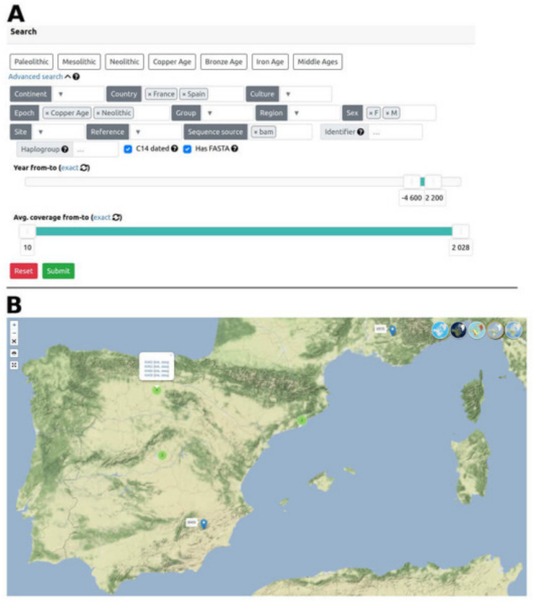
Genomics and bioinformatics: We are equipped with two Illumina sequencers (NextSeq 500 and 2000) and an Oxford Nanopore MinION platform. Since 2019, we have been using single cell transcriptomics and epigenomics technology (10x Genomics) and recently we have established spatially resolved transcriptomics workflow. As a part of our know-how, we develop sophisticated bioinformatical tools and pipelines to analyze produced data sets as well as those available in public databases.

Cancer transcriptomics: We have been involved in a long term-projects focused on head and neck squamous cell carcinoma and skin cancers. We focus on tumor microenvironment and on interactions between cancer associated fibroblasts and malignant cells. We have collected a large data sets of patient samples (>100 patients) and cancer associated fibroblasts that serve as a basis for our studies.

Bioinformatic databases: The laboratory forms the local node of the Pan-European ELIXIR bioinformatics research infrastructure. We curate and maintain the database of mitochondrial sequences coming from the ancient DNA samples, AmtDB, and the database of human endogenous retroviruses, HERVd.

We use all these high-throughput and bioinformatic tools to conduct our own scientific projects, for long term collaborations with our colleagues and clinicians, and to support other scientific groups at the Institute. Indeed, an integral part of our laboratory is the Core Facility of Genomics and Bioinformatics.





Left: We created a simplified spheroid model of melanoma. In this model, melanoma cells interact with photodamaged fibroblasts. The cells clearly divide in different functional classes. Right: A screenshot from the web interface of the AmtDB database. The database is a key platform for ancient population genetic studies.

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LABORATORY OF

GENOME INTEGRITY

DNA damage, cellular senescence, glioma, cancer resistance, PML

Zdeněk Hodný

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Cells with unfinished DNA damage signalling (uDDS) caused by complex or difficult to repair DNA damage (called senescent cells) secrete a diverse set of factors, including pro-inflammatory cytokines and TGF beta family morphogens with multifaceted impact on surrounding tissues. The composition of this secretome, termed senescence-associated secretory phenotype (SASP), is shaped by numerous factors including nature of DNA-damaging stimulus, cell type and metabolic state, and cell-to-cell interaction in surrounding tissues; therefore, the composition of SASP has to be studied for each specific biological condition. The importance of studying SASP to uncover the pathogenesis of human diseases comes from studies including ours demonstrating, for instance, its impact on malignant features of cancer cells such as proliferation, invasiveness and migration, cancer stem cell mobilization, and therapeutic resistance. Analyses of secretomes directly in tissues are currently technically challenging. New methods and approaches have to be developed to decipher the complex information exchange among cells in the organism and its impact on homeostasis.

Our research includes understanding the molecular mechanisms leading to uDDS, cellular responses to uDDS, including phenotypic manifestation of the altered secretory milieu in response to uDDS, and defining molecular targets to develop new therapeutic approaches.

Running projects:

1) Understanding the nature of complex/unrepaired DNA damage

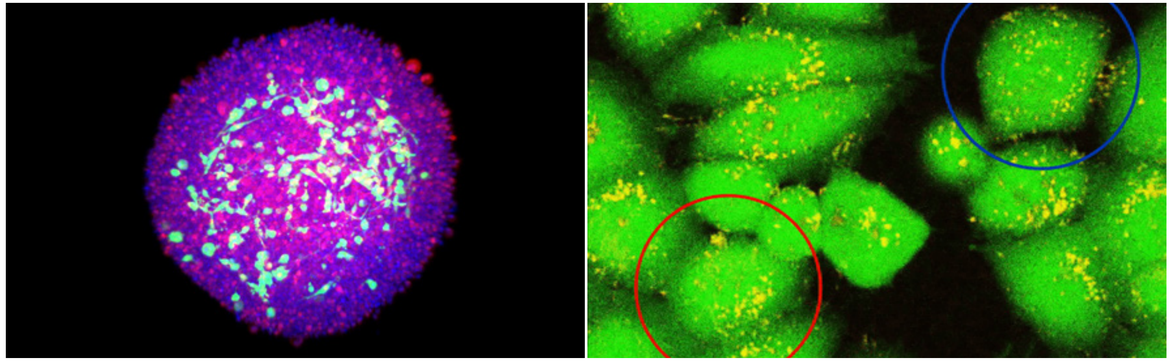
Besides telomeres, ribosomal DNA (rDNA) loci represent repetitive DNA sequences being difficult to repair. We focus on specific mechanisms damaging rDNA loci, their repair, stability and role in tumorigenesis.

2) Role of the tissue microenvironment in glioma therapeutic resistance

High-grade gliomas belong to less treatable human malignant tumours. In cooperation with Karolinska Institute, Sweden, and medical faculties of Masaryk University, Brno and Palacky University, Olomouc, we seek to reveal the molecular mechanisms behind glioma therapeutic resistance.

3) Development of new drugs and nanotherapeutics

In frame of the cooperation network including University Hospital of Hradec Kralove, Czech Technical University in Prague, and Second Faculty of Medicine, Charles University, Prague we are developing new compounds with anti-tumour and anti-aging properties (senolytic and senomodulatory drugs) and nanotechnology-based approaches for cancer treatment.



Left: 3-D glioma spheroid developed as a model to study interactions among cancer (red) and senescent (green) cells (visualized by light sheet microscopy; cell nuclei in blue). Right: Demonstration of thermotherapy by gold nanorods (yellow) in cancer cells induced by near infrared light (920 nm) produced by femto second laser. Cell death by apoptosis (blue circle) and necrosis (red circle) were detected by membrane blebbing and loss of calcein fluorescence (green), respectively.

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4. Rysanek, D., P. Vasicova, J.N. Kolla, D. Sedlak, L. Andera, J. Bartek*, and Z. Hodny*. 2022. Synergism of BCL-2 family inhibitors facilitates selective elimination of senescent cells. Aging. 14:6381-6414.
5. Salovska, B., A. Kondelova, K. Pimkova, Z. Liblova, M. Pribyl, I. Fabrik, J. Bartek, M. Vajrychova*, and Z. Hodny*. 2022. Peroxiredoxin 6 protects irradiated cells from oxidative stress and shapes their senescence-associated cytokine landscape. Redox biology. 49:102212.

LABORATORY OF

CELL DIFFERENTIATION

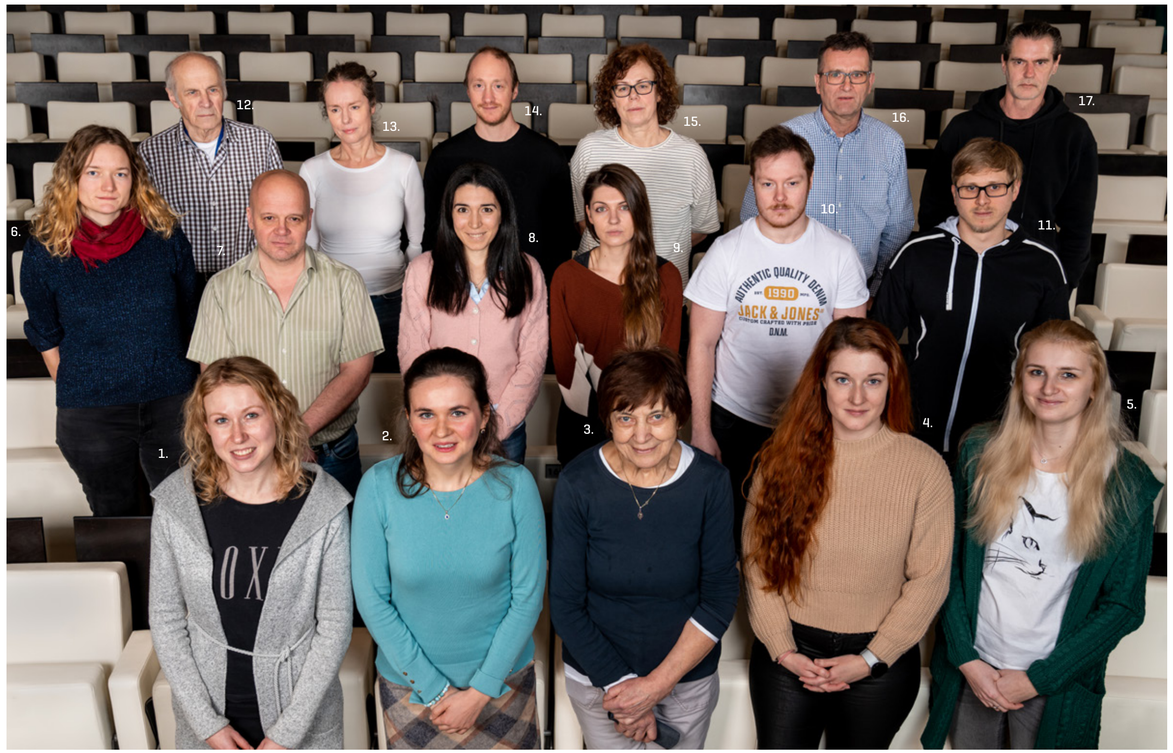
Hematopoietic cell development, signalling pathways, cytokines, small molecules, zebrafish, lamprey, chicken

Petr Bartůněk

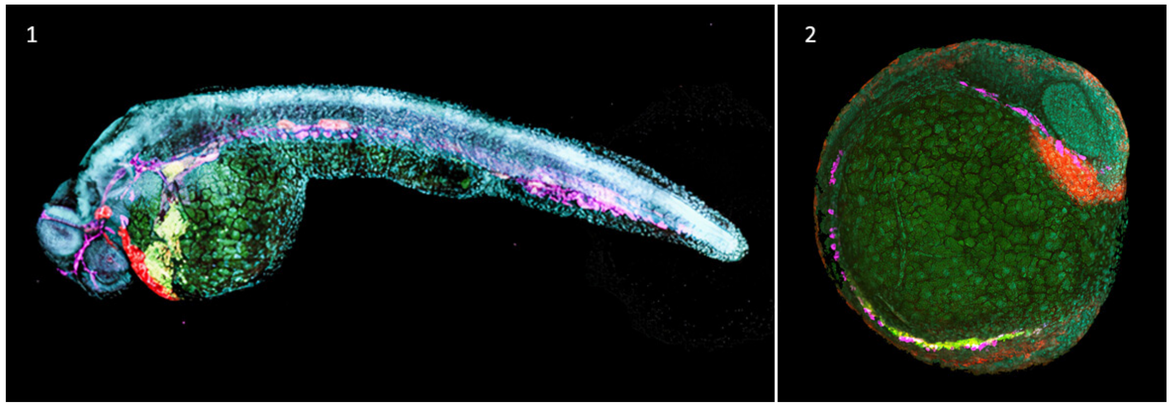
The main interest of the laboratory is to study the molecular mechanism of cell fate determination. We use cytokines/growth factors and small molecules as tools to manipulate hematopoietic cell fate in model organisms (zebrafish, lamprey, chicken, mouse) and human primary cells to gain insight into the mechanisms underlying self-renewal, proliferation and differentiation.

Zebrafish are suitable model organisms for modeling human disease and their small size makes them advantageous for high-throughput preclinical drug screening. We have developed a novel system for bioluminescent detection of transplanted cells that will accelerate the drug development process. For this purpose, we used the NanoLuc luciferase, which provided rapid quantification of tumor cell growth in vivo with high sensitivity and low background compared to conventional fluorescence measurements.

We also focused on M-CSFR/CSF1R signalling and the role of cytokines Csf1a, Csf1b and Il34 in zebrafish embryonic and adult hematopoiesis. We used a set of zebrafish loss-of-function mutants to discern the effects of functional defects in Csf1-receptors and -ligands. We show that Csf1a controls embryonic macrophage expansion and Il34, acting through Csf1rb, is important for embryonic granulopoiesis. We further studied the role of zebrafish Kit ligands in hematopoietic development and performed gain-of-function experiments in zebrafish embryos, which showed that both ligands cooperate with erythropoietin (Epo) in promoting erythroid cell expansion. This was further verified using ex vivo cultures of erythroid progenitors grown in suspension culture or semi-solid media. Thus, our studies clearly demonstrated that hematopoietic cytokine signalling is evolutionarily conserved from fish to humans.



In the picture: 1. Dobiášovská Ivana | 2. Vondráková Zuzana | 3. Dvořáková Marta | 4. Hojerová Tereza | 5. Hingarová Tereza | 6. Jarošová Šárka | 7. Epp Allan Trevor | 8. Jovičić Jovana | 9. Maystorova Rositsa | 10. Blažka Martin | 11. Kovář Martin | 12. Dvořák Michal | 13. Machoňová Olga | 14. Svoboda Ondřej | 15. Zíková Martina | 16. Bartůněk Petr | 17. Schuster Björn



Embryos were fixed at segmentation 8 somites stage (11.5 hpf) or pharyngula stage (30 hpf), respectively and stained using gata1a (green), klf17 (red) and etv2 (magenta) by Hybridisation Chain Reaction probes (HCR, Molecular instruments). The nuclei were co-stained with DAPI (cyan). In figure 1, the gata1a and klf17 positive primitive erythroid progenitors that are differentiating from early mesoderm are located in a caudal region within the inner cell mass and will enter circulation (figure 2) later on. Migrating endothelial vein and arterial progenitors are visualized by etv2 probe (figure 1) and will give rise to blood vessels (figure 2). The klf17 positive structure at the anterior-ventral side of the yolk sac will develop into the hatching gland throughout subsequent development. Imaging was performed using Dragon fly (Andor) spinning disk.

Selected publications:

1. Oltova J, Svoboda O, Machonova O, Svatonova P, Traver D, Kolar M, Bartunek P*. Zebrafish Kit ligands cooperate with erythropoietin to promote erythroid cell expansion. Blood Adv. 2020 Dec 8;4(23):5915-5924.
2. Sedlak D, Wilson TA, Tjarks W, Radomska HS, Wang H, Kolla JN, Leśnikowski ZJ, Alena Spickakova, Ali T, Ishita K, Rakotondraibe LH, Vibhute S, Wang D, Anzenbacher P, Bennett C, Bartunek P*, Coss CC*. Structure-Activity Relationship of para-Carborane Selective Estrogen Receptor β Agonists. J Med Chem 2021 Jul 8;64(13):9330-9353.
3. Hason M, Mikulasova T, Machonova O, Pombinho A, van Ham TJ, Irion U, Nüsslein-Volhard C, Bartunek P*, Svoboda P*. M-CSFR/CSF1R signaling regulates myeloid fates in zebrafish via distinct action of its receptors and ligands. Blood Adv 2022 Mar 8;6(5):1474-1488.
4. Kralova K*, Popr M, Valecka J, Bartunek P. Sterolight as imaging tool to study sterol uptake, trafficking and efflux in living cells. Sci Rep 2022 Apr 15;12(1):6264.
5. Hason M, Jovicic J, Vonkova I, Bojic M, Simon-Vermot T, White RM, Bartunek P*. Bioluminescent Zebrafish Transplantation Model for Drug Discovery. Front Pharmacol 2022 Apr 27;13:893655.

LABORATORY OF

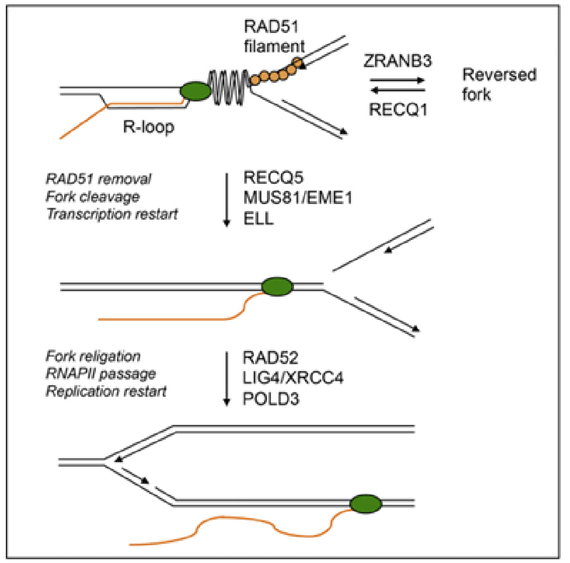
CANCER CELL BIOLOGY

Genome instability, cell cycle checkpoint, replication stress, protein phosphorylation, cancer, cancer predisposition

Libor Macůrek

In the picture: 1. Saha Tias | 2. Aquino Perez Cecilia | 3. Otáhalová Barbora | 4. Macůrek Libor | 5. Elgendy Mohamed | 6. Váníková Lucie | 7. Palek Matouš | 8. Palková Natálie | 9. Huňová Oravetzová Anna | 10. Chalupová Zuzana | 11. Dobrovolná Jana | 12. Gui Wentao | 13. Shukla Kaustubh | 14. Zuev Anton

Integrity of human genome is protected by surveillance mechanisms that coordinate the cell cycle progression and DNA repair. In the presence of DNA damage, cells temporarily arrest in the cell cycle checkpoint to prevent transmission of mutations to progeny and continue proliferation after completing DNA repair. The checkpoint and DNA repair are tightly interconnected by signalling cascades that involve protein phosphorylation (ATM, ATR, CHK1/2, CDK1, PLK1 kinases), ubiquitination (BRCA1, RNF168, 53BP1) and gene expression (tumour suppressor protein p53). Deficient cell cycle checkpoints or impaired DNA repair allow proliferation in the presence of damaged DNA, promoting genome instability and eventually malignant transformation. In our laboratory, we employ cell and molecular biology approaches, CRISPR-mediated gene editing and transgenic mouse models to investigate how cells respond to DNA damage. We also seek for genetic defects in cancer cells that could be exploited for personalized cancer treatment.



Replication stress induces R-loop formation in the cell nucleus.

Topic 1. Role of PPM1D/WIP1 in DNA damage response and oncogenesis

Protein phosphatase PPM1D/Wip1 is an important negative regulator of tumour suppressor p53 and promotes termination of the cell cycle checkpoint. High expression of PPM1D/Wip1 is common in cancer. We identified new truncating mutations in PPM1D/Wip1 that impair the cell cycle checkpoints. Using a transgenic mouse model, we have now confirmed the ability of PPM1D/Wip1 mutations to promote cancer. By combining proteomic approaches, biochemistry and cell/molecular biology, we investigate mechanisms of PPM1D/Wip1 function in human cells and seek for its novel targets at chromatin.

Topic 2. Role of R-loops in genomic instability

R-loops are three-stranded nucleic acid structures generated by invasion of the nascent transcript to the DNA duplex behind the transcription complex. R-loops are emerging as a major source of DNA replication stress and genomic instability. We apply mass proteomic approaches and functional siRNA screens to identify novel factors involved in the metabolism of R-loops and G4 structures and study their relationship to DNA replication. Using these approaches, we have recently identified helicase DDX17 as a new factor involved in resolution of the R-loop-mediated transcription-replication conflicts. We have also described a model for resumption of DNA synthesis after fork stalling that requires cleavage by MUS81, ligation by LIG4 and active transcription by elongation factor ELL.

Topic 3. New proteins involved in the cell cycle and mitosis

By expression profiling in human cells, we have identified several new regulators of the cell cycle and mitosis. We found that depletion of FAM110A impaired chromosomal alignment in mitosis and resulted in chromosomal defects. FAM110A localizes at poles of the mitotic spindle and its function depends on phosphorylation by casein kinase 1.

Topic 4. Identification of new cancer-predisposing genes

DNA repair and checkpoint genes are typical tumour suppressors that are commonly inactivated in human cancers. When present in the germline, these mutations increase a risk of cancer development in affected families (such as BRCA1 or CHEK2 in familial breast cancer). In collaboration with medical geneticists, we develop cell-based assays for functional evaluation of newly identified mutations which will allow better prevention of familial cancers.

Selected publications:

1. Martinikova AS, Burocziova M, Stoyanov M, Macurek L*. Truncated PPM1D Prevents Apoptosis in the Murine Thymus and Promotes Ionizing Radiation-Induced Lymphoma. Cells 2020; 9(9):2068.
2. Aquino Perez C, Burocziova M, Jenikova G, Macurek L*. CK1-mediated phosphorylation of FAM110A promotes its interaction with mitotic spindle and controls chromosomal alignment. EMBO Rep. 2021; 22(7):e51847
3. Chappidi N, Nascakova Z, Boleslavska B, Zellweger R, Isik E, Andrs M, Menon S, Dobrovolna J, Balbo Pogliano C, Matos J, Porro A, Lopes M, Janscak P*. Fork Cleavage-Religation Cycle and Active Transcription Mediate Replication Restart after Fork Stalling at Co-transcriptional R-Loops. Mol Cell. 2020; 77(3):528-541.e8.
4. Boleslavska B, Oravetzova A, Shukla K, Nascakova Z, Ibini ON, Hasanova Z, Andrs M, Kanagaraj R, Dobrovolna J, Janscak P*. DDX17 helicase promotes resolution of R-loop-mediated transcription-replication conflicts in human cells. Nucleic Acids Res. 2022; 50(21):12274-12290.

LABORATORY OF

LEUKOCYTE SIGNALLING

Leukocyte signal transduction, inflammation, immune response, autoinflammatory diseases, membrane adaptor proteins

Tomáš Brdička

In the picture: 1. Pavliuchenko Nataliia | 2. Durić Iris | 3. Brdička Tomáš | 4. Angelisova Pavla | 5. Skopcová Tereza

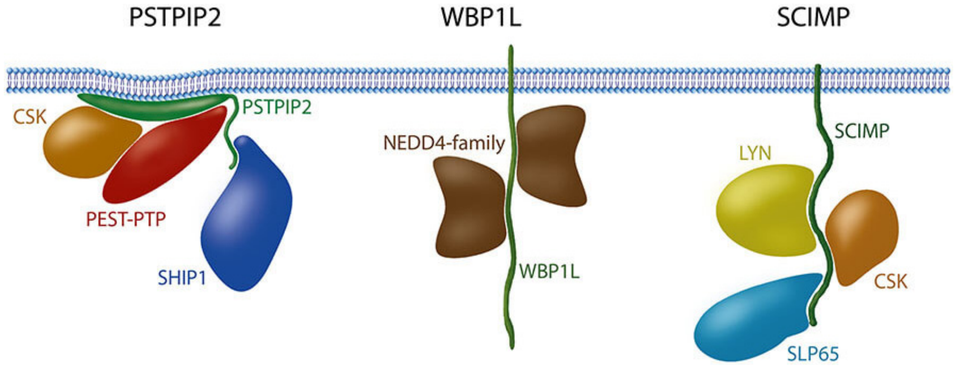
The Laboratory of Leukocyte Signalling is studying the molecular mechanisms of how various leukocyte proteins regulate signal transduction by surface receptors and how their dysfunction triggers disease. Within this relatively broad field, our research focuses mainly on membrane adaptor proteins and Src-family kinases (SFK) and on their roles in inflammation and haematopoiesis.

Membrane adaptor proteins are membrane-associated proteins, which are responsible for organizing networks of signalling molecules near cellular membranes. Some of these proteins have key roles in propagation of the signal generated by leukocyte surface receptors, while others have important regulatory functions. One of the most interesting membrane adaptors is known as PSTPIP2. Its deficiency in the mouse model leads to the development of chronic multifocal osteomyelitis. It is an autoinflammatory disease characterized by spontaneous bone and skin inflammation, which closely resembles several human disorders. We are studying the molecular mechanisms of how the complex of proteins organized by PSTPIP2 in neutrophil granulocytes regulates inflammatory processes and how its absence leads to impaired control of inflammation. Our most interesting discoveries include the finding that dysregulated reactive oxygen species production by NADPH oxidase is specifically important for inflammatory bone damage in this disease and that the disease progression is very likely dependent on SFK and the receptors they regulate.

Another membrane adaptor protein we are extensively studying is WBP1L, aberrantly expressed in certain types of childhood leukaemia and potentially affecting the treatment outcome. We have found that WBP1L regulates haematopoiesis, in part via regulation of several key cytokine/chemokine receptors in haematopoietic stem and progenitor cells. We are currently analysing the molecular mechanisms of this regulation and its effects on the biology of the haematopoietic system.

Apart from membrane adaptors, we are also investigating the roles of Src-family kinases in leukocyte signalling and disease. Within this topic, we are analysing their roles in the regulation of antigen receptor signalling and mutations in their genes causing diseases in humans.

Finally, part of our team engages in research of the methods of detergent-free membrane disintegration and their utilization in the analysis of organization of plasma membrane lipids, membrane adaptors, and Src-family kinases into membrane nanodomains.



Examples of molecular signalling complexes organized by leukocyte membrane adaptors PSTPIP2, WBP1L, and SCIMP at cellular membranes.

Selected publications:

1. Pavliuchenko N, Duric I, Kralova J, Fabisik M, Spoutil F, Prochazka J, Kasparek P, Pokorna J, Skopcova T, Sedlacek R, and Brdicka T*: Molecular interactions of adaptor protein PSTPIP2 control neutrophil-mediated responses leading to autoinflammation. Front Immunol 2022 13: 1035226.
2. Kralova J, Pavliuchenko N, Fabisik M, Ilievova K, Spoutil F, Prochazka J, Pokorna J, Sedlacek R, and Brdicka T*: The receptor-type protein tyrosine phosphatase CD45 promotes onset and severity of IL-1β-mediated autoinflammatory osteomyelitis. J Biol Chem 2022 297: 101131
3. Kanderova V, Svobodova T, Borna S, Fejtkova M, Martinu V, Paderova J, Svaton M, Kralova J, Fronkova E, Klocperk A, Pruhova S, Lee-Kirsch MA, Hornofova L, Koblizek M, Novak P, Zimmermannova O, Parackova Z, Sediva A, Kalina T, Janda A, Kayserova J, Dvorakova M, Macek M, Pohunek P, Sedlacek P, Poh A, Ernst M, Brdicka T*, Hrusak O*, and Lebl J*: Early-onset pulmonary and cutaneous vasculitis driven by constitutively active SRC-family kinase HCK. J Allergy Clin Immunol 2021 149(4): 1464-1472
4. Kralova J, Drobek A, Prochazka J, Spoutil F, Fabisik M, Glatzova D, Borna S, Pokorna J, Skopcova T, Angelisova P, Gregor M, Kovarik P, Sedlacek R, and Brdicka T*: Dysregulated NADPH Oxidase Promotes Bone Damage in Murine Model of Autoinflammatory Osteomyelitis. J Immunol 2020 204: 1607-1620
5. Borna S, Drobek A, Kralova J, Glatzova D, Splichalova I, Fabisik M, Pokorna J, Skopcova T, Angelisova P, Kanderova V, Starkova J, Stanek P, Matveichuk OV, Pavliuchenko N, Kwiatkowska K, Protty MB, Tomlinson MG, Alberich-Jorda M, Korinek V, and Brdicka T*: Transmembrane adaptor protein WBP1L regulates CXCR4 signalling and murine haematopoiesis. J Cell Mol Med 2020 24: 1980-1992

LABORATORY OF

HAEMATOONCOLOGY

Haematology, haematopoietic stem cells, neutrophilic differentiation, granulocytes, emergency granulopoiesis, inflammation

Meritxell Alberich Jordà

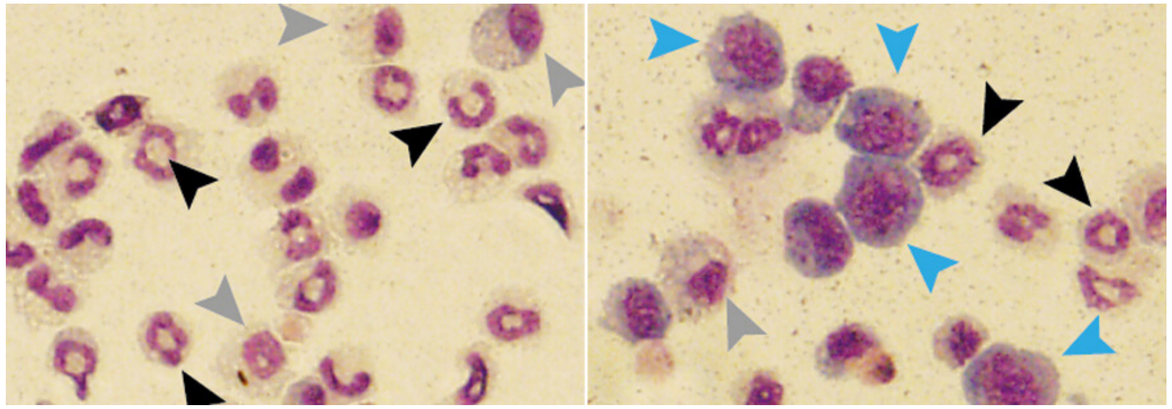
In our research group, we investigate the regulation of haematopoietic stem cell (HSC) maintenance and fate by cell intrinsic as well as cell extrinsic factors. On one hand we focus on transcription factors and their target genes, determine whether these elements are altered in human leukaemias (in particular acute myeloid leukaemia, AML), and elucidate their contribution to leukaemogenesis. On the other hand, we investigate how cell extrinsic factors, mainly originated in inflammatory conditions, regulate HSC self-renewal and how they impact myeloid commitment.

Our three main research lines are:

1. To determine the function of C/EBPa target genes in normal and malignant haematopoiesis.
2. To define the role of the b-catenin-TCF/LEF transcription-mediating complex in normal and aberrant haematopoiesis.
3. To assess the effects of inflammation/infection in HSC fitness, myeloid differentiation, and leukaemogenesis.

To reach these goals, we employ murine and human primary cells, as well as murine models. We perform a variety of in vitro assays to assess cell proliferation, apoptosis, colony-forming potential, re-plating ability, differentiation, and migration. Further, we carry out murine bone marrow cell transplantation assays, challenge mice with infectious agents, and perform HSC mobilization assays in vivo. Using primary cells from patients suffering from AML, we generate PDX models. To get novel insights into the molecular mechanism of stem cell regulation and transformation, we employ molecular biology approaches including RNA-seq, ATAC-seq and ChIP-seq/qPCR.
Together, we aim at understanding the mechanisms that control HSC maintenance and fate, and determine cell intrinsic and extrinsic factors that contribute to myeloid commitment and differentiation. Ultimately, our work will contribute to establishing knowledge for the development of better AML therapies.

In the picture: 1. Kuzmina Maria | 2. Alberich Jorda Meritxell | 3. Vaníčková Karolína | 4. Kosanović Sladana | 5. Ribeiro Bas Irina | 6. Burócziová Monika | 7. Adamcová Miroslava Kari | 8. Grušanović Srdjan | 9. Shaikh Mehak Nihal



Cell morphological analysis assessed on May-Grunwald Giemsa stained cytospins. The left image shows healthy mature granulocytes (black arrows). The right image shows blast cells that were unable to differentiate towards mature granuloctyes (blue arrows). Cells were isolated from cultures favouring growth of murine myeloid cells.

Selected publications:

1. Danek P, Kardosova M, Janeckova L, Karkoulia E, Vanickova K, Fabisik M, Lozano Asencio C, Benoukraf T, Tirado-Magallanes R, Zhou Q, Burocziova M, Rahmatova S, Pytlik R, Brdicka T, Tenen DG, Korinek V, Alberich Jorda M*: β-catenin-TCF/LEF signaling promotes steady-state and emergency granulopoiesis via G-CSF receptor upregulation. Blood 2020 Nov 7:e54729
2. Lobo de Figueiredo-Pontes L, Adamcova MK, Grusanovic S, Kuzmina M, Aparecida Lopes I, Fernandes de Oliveira Costa A, Zhang H, Strnad H, Lee S, Moudra A, Jonasova AT, Zidka M, Welner RS, Tenen DG*, Alberich-Jorda M*: Improved hematopoietic stem cell transplantation upon inhibition of natural killer cell-derived interferon-gamma. Stem Cell Reports 2021 16(8): 1999-2013.
3. Stavast CJ, van Zuijen I, Karkoulia E, Özçelik A, van Hoven-Beijen A, Leon LG, Voerman JSA, Janssen GMC, van Veelen PA, Burocziova M, Brouwer RWW, van IJcken WFJ, Maas A, Bindels EM, van der Velden VHJ, Schliehe C, Katsikis PD, Alberich-Jorda M, Erkeland SJ: The tumor suppressor MIR139 is silenced by POLR2M to promote AML oncogenesis. Leukemia 2022 36(3): 687-700.
4. Lobo de Figueiredo-Pontes L, Adamcova MK, Welner RS, Tenen DG*, Alberich-Jorda M*: Response to NK cell content does not seem to influence engraftment in ex vivo T cell depleted haploidentical stem cell transplantation. Stem Cell Reports 2022 17(3): 446-447.
5. Grusanovic S, Danek P, Kuzmina M, Adamcova MK, Burocziova M, Mikyskova R, Vanickova K, Kosanovic S, Pokorna J, Reinis M, Brdicka T, Alberich-Jorda M*: Chronic inflammation decreases HSC fitness by activating the druggable Jak/Stat3 signaling pathway. EMBO Rep 2022, e54729

LABORATORY OF

CELL MOTILITY

Flagellum/cilium, microtubule-based cytoskeleton, kinetoplastid parasites, mammalian cells, advanced light and electron microscopy

Vladimír Varga

In the picture: 1. Zelená Marie | 2. Pavlisková Hana | 3. Penkovska Kateryna | 4. Varga Vladimír | 5. Štěpánek Luděk

In the Laboratory of Cell Motility, we study the eukaryotic flagellum and cilium (the terms are interchangeable), a fascinating organelle with motile, signalling and sensory roles. The flagellum/cilium is evolutionarily well conserved and is present on surfaces of many eukaryotic cells, including important parasitic protists and most mammalian cell types. In humans, malfunction of cilia causes severe hereditary disorders called ciliopathies.

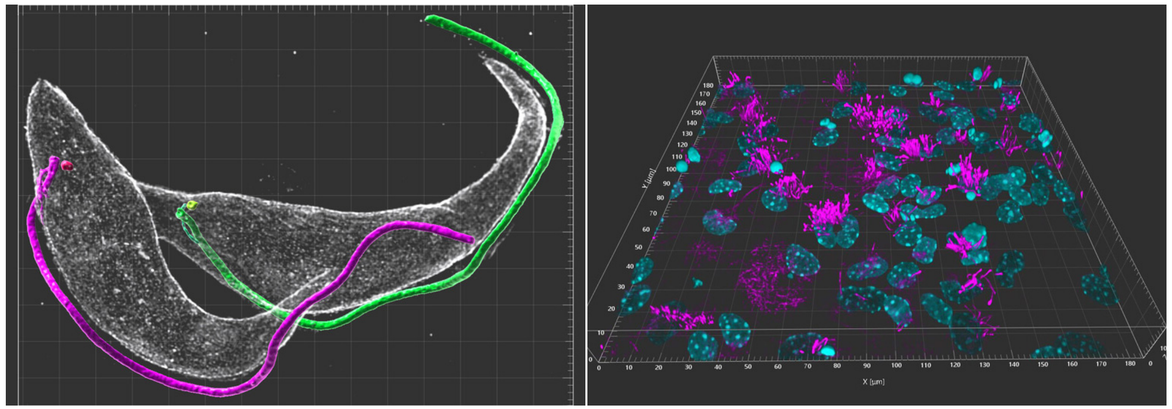
The principal structural and force-generating component of the flagellum/cilium is the microtubule-based axoneme. The axoneme consists of several hundred protein species organized in a highly defined manner. How does the cell form such a complex yet highly organized structure?

Using the highly experimentally tractable flagellated protist *Trypanosoma brucei*, the causative agent of human African trypanosomiasis, we have developed and optimized biochemical, cell biology, and imaging techniques to identify proteins critical for the processes of axonemal construction and length regulation.

Importantly, due to the high evolutionary conservation of the organelle, we were able to identify mammalian orthologues of some of these proteins and assess their roles in mammalian cells.

Finally, to reveal intrinsic activities of these newly identified proteins, we study their behaviour by in vitro biochemical assays. In particular, we employ microscopy-based single-molecule assays, which provide deep mechanistic insights into the activities of individual molecules.

We believe that integrating these approaches will provide a comprehensive understanding of the processes orchestrating the axonemal growth and will give insights into the causes of certain ciliopathies.



Left: The microtubule-based cytoskeleton of a *Trypanosoma brucei* cell. Flagella and structures associated with their base are highlighted in colour. The cell was imaged using the expansion microscopy approach. Right: Cell culture of mouse multiciliated ependymal cells. This type of cells is found of the surface of the ventricular system in the brain, and the ciliary beating contributes to generation of the cerebrospinal fluid flow. The cilia are in magenta, cell nuclei in cyan.

Selected publications:

1. Gorilak P, Pružincová M, Vachova H, Olšinová M, Schmidt Cernohorska M, Varga V*: Expansion microscopy facilitates quantitative super-resolution studies of cytoskeletal structures in kinetoplastid parasites. Open Biol 2021 11(9): 210131.
2. Kiesel P, Alvarez Viar G, Tsoy N, Maraspini R, Gorilak P, Varga V, Honigmann A, Pigino G: The molecular structure of mammalian primary cilia revealed by cryo-electron tomography. Nat Struct Mol Biol 2020 Dec;27(12):1115-1124.
3. Vachova H, Alquicer G, Sedinova M, Sachova J, Hradilova M, Varga V*: A rapid approach for in locus overexpression of *Trypanosoma brucei* proteins. Mol Biochem Parasitol 2020 239: 111300.

LABORATORY OF

ADAPTIVE IMMUNITY

Immunity, T cells, signalling, disease models

Ondřej Štěpánek

In the picture: 1. Prasai Avishek | 2. Cupák Ladislav | 3. Uleri Valeria | 4. Andreyeva Arina | 5. Kratochvílová Anna | 6. Janušová Šárka | 7. Mašková Kristýna | 8. Paprčková Darina | 9. Cesneková Michaela | 10. Neuwirth Aleš | 11. Sprague Carly | 12. Tsyklauri Oksana | 13. Štěpánek Ondřej | 14. Huranová Martina | 15. Ivashchenko Olha | 16. Cimermanová Veronika | 17. Niederlová Veronika | 18. Michálik Juraj

We consider T cells as the most fascinating cells in our bodies. Unlike the vast majority of other somatic cells, each T cell is genetically unique, because it shuffles the pieces of DNA that encode its antigenic receptor. It means that each single T cell has its unique antigenic receptor with a unique specificity. We can compare the T cells to an army of soldiers, each of them carrying a unique weapon used in a specific situation. When the organism is infected with a pathogen, there are always a couple of T cells with the right weapon/receptor that initiate the adaptive immune response. On the other hand, too much of T-cell reactivity might induce friendly fire, or autoimmunity in immunological terms.

We elucidate how T cells make the proper fate decisions to elicit a potent immune protection and maintain self-tolerance at the same time. Our current research projects focus on the mechanisms of T-cell signalling via antigenic and germ-line encoded receptors, characterization of particular T-cell subsets, and mechanisms of signalling induced by a prominent T-cell cytokine, IL-17. Moreover, we study the biology of a protein complex called BBSome in immune cells and ciliated cells, to understand a rare disease called Bardet-Biedl Syndrome.

Impact of antigenic exposure on the immune system

Selected publications:

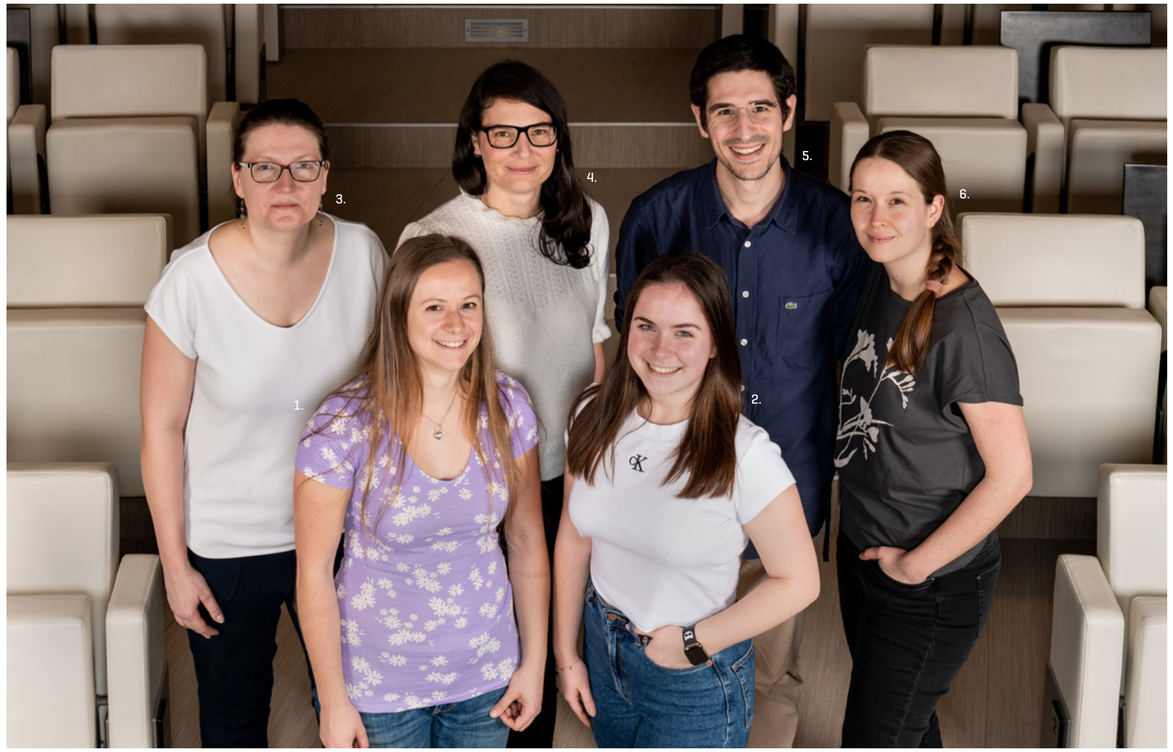
1. Knizkova D, Pribikova M, Draberova H, Semberova T, Trivic T, Synackova A, Ujevic A, Stefanovic J, Drobek A, Huranova M, Niederlova V, Tsyklauri O, Neuwirth A, Tureckova J, Stepanek O*, Draber P*: CMTM4 is a subunit of the IL-17 receptor and mediates autoimmune pathology. Nat Immunol 2022 23: 1644–1652
2. Paprckova D, Niederlova V, Moudra A, Drobek A, Pribikova M, Janusova S, Schober K, Neuwirth A, Michalik J, Huranova M, Horkova V, Cesnekova M, Simova M, Prochazka J, Balounova J, Busch DH, Sedlacek R, Schwarzer M, Stepanek O*: Self-reactivity of CD8 T-cell clones determines their differentiation status rather than their responsiveness in infections. Front Immunol 2022 13: 1009198.
3. Tsyklauri O, Niederlova V, Forsythe E, Prasai A, Drobek A, Kasparek P, Sparks K, Trachtulec Z, Prochazka J, Sedlacek R, Beales P, Huranova M*, Stepanek O*: Bardet-Biedl Syndrome ciliopathy is linked to altered hematopoiesis and dysregulated self-tolerance. EMBO Rep 2021, e50785.
4. Draberova H, Janusova S, Knizkova D, Semberova T, Pribikova M, Ujevic A, Harant K, Knapkova S, Hrdinka M, Fanfani V, Stracquadanio G, Drobek A, Ruppova K, Stepanek O*, Draber P*: Systematic analysis of the IL-17 receptor signalosome reveals a robust regulatory feedback loop. EMBO J 2020, e104202.
5. Horkova V, Drobek A, Mueller D, Gubser C, Niederlova V, Wyss L, King CG, Zehn D, Stepanek O*: Dynamics of the Coreceptor-LCK Interactions during T Cell Development Shape the Self-Reactivity of Peripheral CD4 and CD8 T Cells. Cell Rep 2020 30(5): 1504-1514.e7.

LABORATORY OF

GENOME DYNAMICS

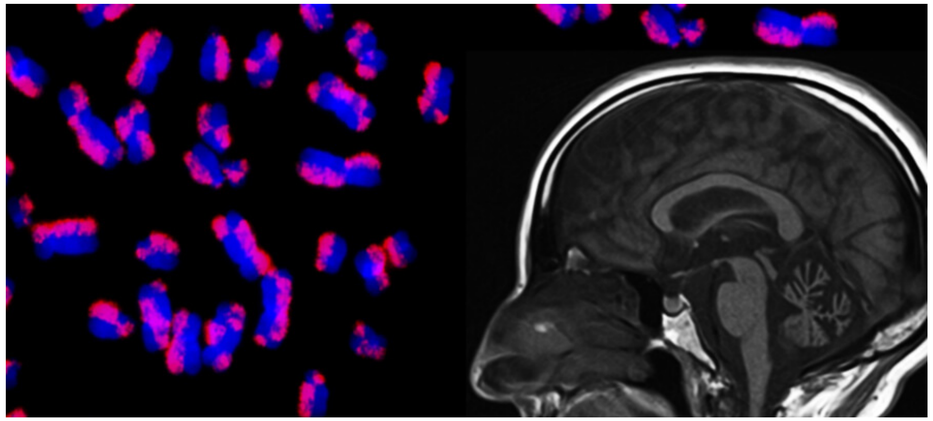
DNA single-strand breaks, ADP-ribosylation, RNA metabolism, neurological disease

Hana Hanzlíková



In the picture: 1. Ilievová Kristýna | 2. Bronišová Denisa | 3. Burdová Kamila | 4. Hanzlíková Hana | 5. Pizarro Madureira Salgado De Oliveira Gonçalo | 6. Cihlářová Zuzana

ADP-ribosylation is a ubiquitous transient post-translational modification of proteins that is involved in a number of major cellular and biological processes, including DNA damage repair, cell proliferation and differentiation, metabolism, stress and immune responses. The main research of our group is focused on ADP-ribosyl transferases; a class of DNA repair enzymes that detect DNA single-strand breaks (SSBs) and signal their presence by catalysing the rapid synthesis of mono(ADP-ribose) and poly(ADP-ribose) and hydrolases; enzymes that catalyse the removal of specific ADP-ribosyl modifications from proteins. SSBs are amongst the most frequent DNA lesions arising in cells that might interfere with RNA processing and transcription and if not repaired correctly can threaten both genetic stability and cell survival. Notably, defects in DNA SSB repair, ADP-ribose metabolism, RNA processing and transcription regulation are associated with hereditary neurodevelopmental and neurodegenerative diseases in human, underscoring the particular importance of these processes in long-lived post-mitotic neurons. We investigate the molecular mechanisms by which DNA SSBs are detected and repaired and we are especially interested in identifying and characterising the protein factors and pathways that couple aberrant ADP-ribose metabolism to neurodegenerative disease. We aim to examine whether the deregulated ADP-ribose metabolism at sites of SSBs extends beyond rare DNA repair-defective diseases to dementia, a neurodegenerative disease that presents the greatest threat to normal human ageing and human health. The risk of being affected by a neurodegenerative disease increases dramatically with age. Although treatments may help relieve some of the physical or mental symptoms associated with neurodegenerative diseases, there are currently no known cures. Therefore, there is a critical need to improve our understanding of what causes neurodegeneration and to develop new approaches for treatment and prevention. The cause of neurodegenerative disorders is often genetic; however, the involved genes and the underlying mechanisms are increasingly diverse, indicating the complexity of brain development and growth. Ultimately, we envisage that our work will lead to new therapeutic avenues for clinical treatment of human neurodegenerative disease.



Left: Increased sister chromatid exchange, the exchange of genetic material between two identical sister chromatids, in cells from a patient suffering from neurodegeneration with defects in the single-strand break repair pathway. Right: Magnetic Resonance Imaging (MRI) scan showing a patient suffering from neurodegeneration with cerebellar atrophy.

Selected publications:

1. Cihlarova Z, Kubovciak J, Sobol M, Krejcikova K, Sachova J, Kolar M, Stanek D, Barinka C, Yoon G, Caldecott KW, Hanzlikova H*: BRAT1 links Integrator and defective RNA processing with neurodegeneration. Nat Commun 2022 13(1): 5026.
2. Vaitsiankova A, Burdova K, Sobol M, Gautam A, Benada O, Hanzlikova H*, Caldecott KW*: PARP inhibition impedes the maturation of nascent DNA strands during DNA replication. Nat Struct Mol Biol 2022 29(4):329-338.
3. Wu W, Hill SE, Nathan WJ, Paiano J, Callen E, Wang D, Shinoda K, van Wietmarschen N, Colón-Mercado JM, Zong D, De Pace R, Shih HY, Coon S, Parsadanian M, Pavani R, Hanzlikova H, Park S, Jung SK, McHugh PJ, Canela A, Chen C, Casellas R, Caldecott KW*, Ward ME*, Nussenzweig A*: Neuronal enhancers are hotspots for DNA single-strand break repair. Nature 2021 593(7859): 440-444.
4. Hanzlikova H*, Prokhorova E, Krejcikova K, Cihlarova Z, Kalasova I, Kubovciak J, Sachova J, Hailstone R, Brazina J, Ghosh S, Cirak S, Gleeson JG, Ahel I, Caldecott KW*: Pathogenic ARH3 mutations result in ADP-ribose chromatin scars during DNA strand break repair. Nat Commun 2020 11(1): 3391.
5. Kalasova I, Hailstone R, Bublitz J, Bogantes J, Hofmann W, Leal A, Hanzlikova H*, Caldecott KW*: Pathological mutations in PNKP trigger defects in DNA single-strand break repair but not DNA double-strand break repair. Nucleic Acids Res 2020 48(12): 6672-6684.

LABORATORY OF

INTEGRATIVE BIOLOGY

Mechanobiology, cytoskeleton, cytolinkers, cell junctions, simple epithelia

Martin Gregor

In the picture: 1. Gemperle Jakub | 2. Ojha Srikant | 3. Sarnová Lenka | 4. Gregor Martin | 5. Outlá Zuzana | 6. Suresh Hiremath Spoorthi | 7. Maninová Miloslava | 8. Kosla Jan | 9. Bisht Piyush | 10. Jiroušková Markéta | 11. Přechová Magdalena | 12. Korelová Kateřina

In the past five years, our main research interests have been:

1. cytoskeleton-dependent regulation of cell-cell contacts in simple epithelia;
2. regulation of cell-matrix adhesions; and
3. cytoskeleton and adhesion-mediated signalling in epithelial-mesenchymal transition, cell migration and invasiveness.

We mainly focus on cytoskeletal linker proteins, in particular plectin, and we study the functional consequences of cytoskeletal organization in cell/tissue mechanics and mechanotransduction, i.e., conversion of physical cues into intracellular mechanosignalling pathways. To fulfil our aims in the complexity of biological systems, we use a combination of in vitro (primary cells and CRISPR/Cas9-targeted cell lines) and in vivo (transgenic models) approaches. Besides conventional molecular biology techniques, we also employ methods that enable us to measure and apply physiologically relevant forces and deformations, such as traction force and atomic force microscopy, magnetic tweezer rheology, cell stretching, and FRET-based tension sensors.

As the Laboratory of Integrative Biology team was established as part of BIOCEV (in January 2015), our long-term interest is also defined by BIOCEV Project 1.1.4.: „Mouse models for studying of physiology and pathophysiology of digestive epithelia" (the Functional Genomics Programme).

This aim has the following core project objectives:

1. identification of genes with unique and essential functions in simple epithelia;
2. generation of mouse models with targeted selected genes; and
3. phenotypic characterization of generated mouse models addressing gene functions in healthy and diseased simple epithelia.

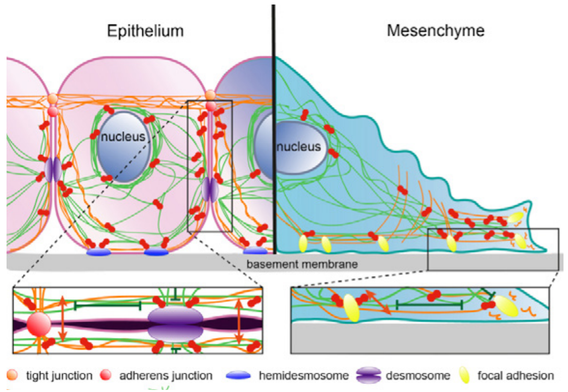


Figure 1. Schematic overview of plectin localization and plectin-mediated crosslinking/anchoring in epithelial (left) and mesenchymal (right) cells.

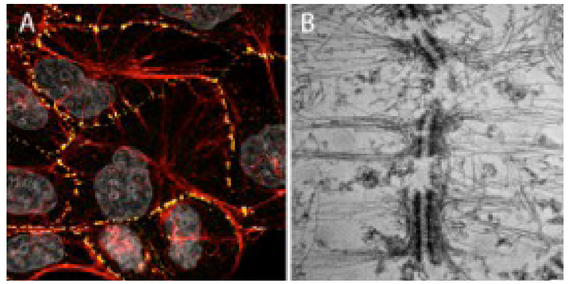


Figure 2. (A) Defective organization of keratin filaments (red) and desmosomes (yellow) in epithelial cells with an inactivated gene encoding the binding protein plectin. (B) Non-functioning desmosomes and keratin filaments imaged by transmission electron microscopy.

Selected publications:

1. Prechova M, Adamova Z, Schweizer AL, Maninova M, Bauer A, Kah D, Meier-Menches SM, Wiche G, Fabry B, Gregor M*: Plectin-mediated cytoskeletal crosstalk controls cell tension and cohesion in epithelial sheets. J Cell Biol 2022 221(3):e202105146.
2. Gerckens M, Schorpp K, Pelizza F, Wögrath M, Reichau K, Ma H, Dworsky AM, Sengupta A, Stoleriu MG, Heinzelmann K, Merl-Pham J, Irmler M, Alsafadi HN, Trenkenschuh E, Sarnova L, Jirouskova M, Frieß W, Hauck SM, Beckers J, Kneidinger N, Behr J, Hilgendorff A, Hadian K, Lindner M, Königshoff M, Eickelberg O, Gregor M, Plettenburg O, Yildirim AÖ, Burgstaller G*: Phenotypic drug screening in a human fibrosis model identified a novel class of antifibrotic therapeutics. Sci Adv 2021 7(52):eabb3673.
3. Krausova A, Buresova P, Sarnova L, Oyman-Eyrilmez G, Skarda J, Wohl P, Bajer P, Sticova E, Bartonova L, Pacha J, Koubkova G, Prochazka J, Spörrer M, Dürrbeck Ch, Stehlikova Z, Vit M, Ziolkowska N, Sedlacek R, Jirak D, Kverka M, Wiche G, Fabry B, Korinek V, Gregor M*: Plectin Ensures Intestinal Epithelial Integrity and Protects Colon Against Colitis. Mucosal Immunol 2021 14:691.
4. Strouhalova K, Přechová M, Gandalovičová A, Brábek J, Gregor M* , Rosel D*: Vimentin Intermediate Filaments as Potential Target for Cancer Treatment. Cancers. 2020 12(1). pii: E184.

LABORATORY OF

CANCER BIOLOGY

Proteasome, cancer, protein degradation, cell cycle, survival

Lukáš Čermák

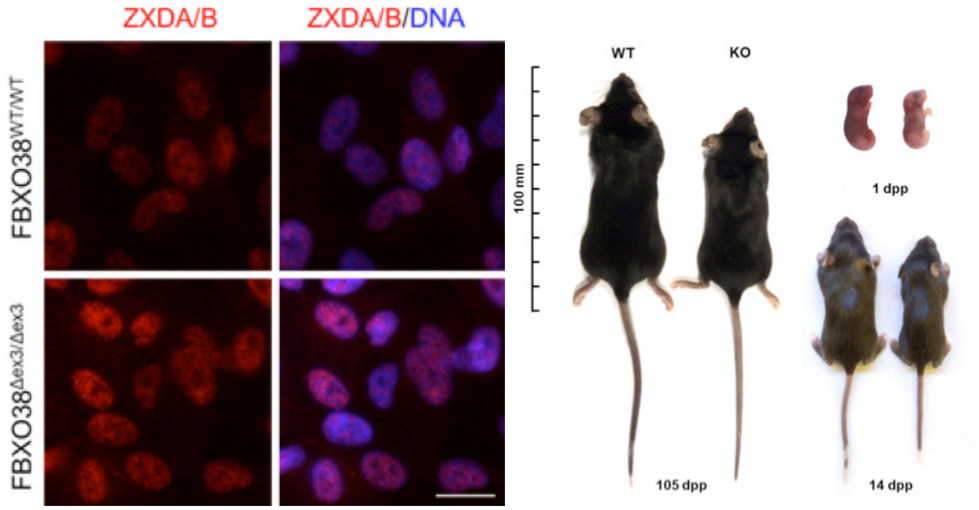
In the picture: 1. Alikhan Abdirov | 2. Kolářová Karolina | 3. Dibus Nikol | 4. Monleón Mario Adrián Martínez | 5. Čermák Lukáš

The primary function of the ubiquitin-proteasome system (UPS) is to degrade unnecessary or damaged proteins. Cullin-RING (CRL) ubiquitin ligases mediate the ubiquitination of many substrates. Our research focuses on discovering novel CRL substrates involved in cancer progression, stress response, or cell cycle. We anticipate that these novel interactions could serve as potential therapeutic targets in cancer and other pathological conditions. We are currently investigating the function of several CRL complexes.

Our main methodological approaches are:

- Biochemical analysis of CRL complexes and the discovery of their substrates and the signalling pathways that control their activity.
- Physiological aspects of CRLs function in the context of mammalian development.

In detail, we investigated the function of the F-box-containing protein 38 (FBXO38). FBXO38 is a substrate receptor for the SKP1-CUL1-dependent ubiquitin ligase (SCF). Several mutations in the FBXO38 gene have been found in patients with early distal hereditary motor neuronopathy, suggesting a role in nervous system homeostasis. Interestingly, another mutation was discovered when studying identical twins with discordant development of gender dysphoria. We identified ZXDA/B zinc finger proteins as its substrates. We further showed that ZXDA/B proteins are responsible for stabilizing centromeric chromatin and that FBXO38 negatively controls this process. Moreover, we investigated the physiological role of FBXO38 during mouse development. We found that FBXO38 controls the growth of several organs, including the testes, where it is expressed by Sertoli cells. Sertoli cells lacking FBXO38 exhibited impaired maturation, leading to improper stimulation of spermatogonia and impaired sperm production. This pathological process was accompanied by stabilization of the FBXO38 substrate ZXDB and changes in CENP-A/B positive centromeric regions.



(A) ZXDA/B is stabilized in FBXO38 KO cells. Wild-type (WT) or FBXO38 knockout RPE-1 cells (FBXO38•ex3/•ex3; KO) were grown on slides, fixed, and immunostained with the ZXDA/B antibody. DNA was stained with DAPI. Scale bar, 20 μm.
(B) Fbxo38 controls growth. Representative images of Fbxo38 WT and KO littermate males of indicated age. Dpp; days postpartum.

Selected publications:

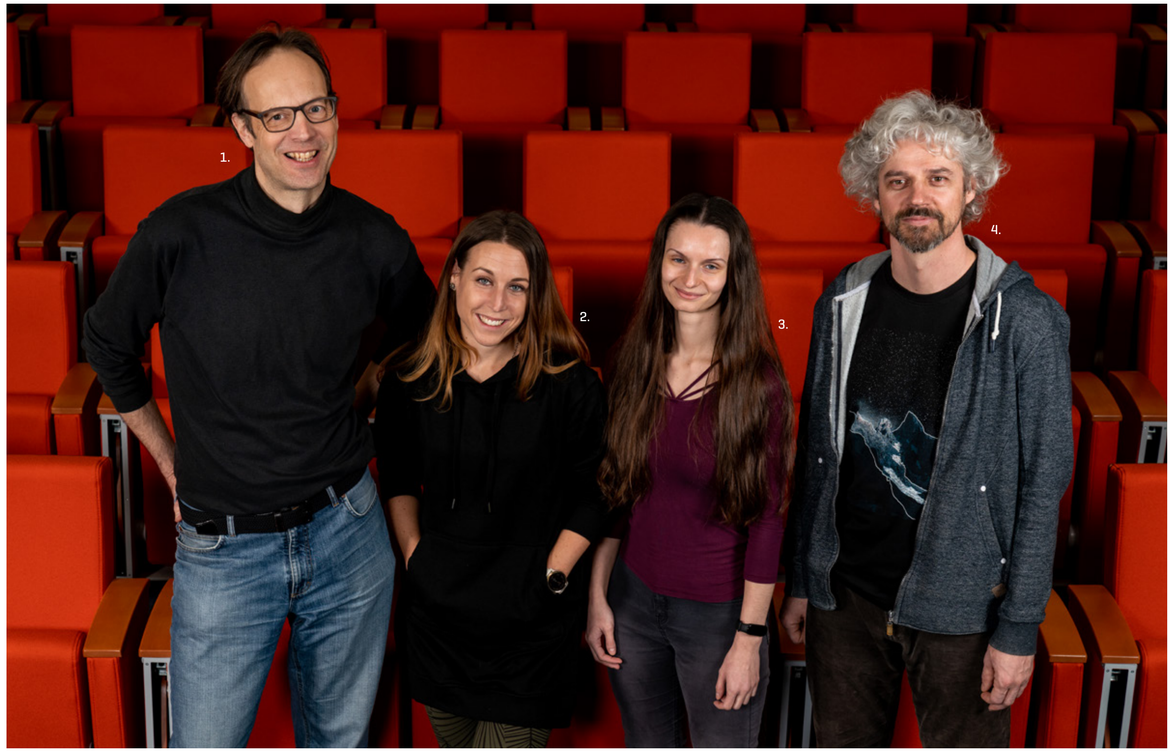
1. Dibus N, Korinek V, Cermak L*. FBXO38 Ubiquitin Ligase Controls Centromere Integrity via ZXDA/B Stability. Front Cell Dev Biol. 2022 Jun 23;10:929288. doi: 10.3389/fcell.2022.929288. eCollection 2022. PMID: 35813202
2. Dibus N, Zobalova E, Monleon MAM, Korinek V, Filipp D, Petrusova J, Sedlacek R, Kasparek P, Cermak L*. FBXO38 Ubiquitin Ligase Controls Sertoli Cell Maturation. Front Cell Dev Biol. 2022 Jun 13;10:914053. doi: 10.3389/fcell.2022.914053. eCollection 2022. PMID: 35769260
3. Lidak T, Baloghova N, Korinek V, Sedlacek R, Balounova J, Kasparek P, Cermak L*. CRL4-DCAF12 Ubiquitin Ligase Controls MOV10 RNA Helicase during Spermatogenesis and T Cell Activation. Int J Mol Sci. 2021 May 20;22(10):5394. doi: 10.3390/ijms22105394. PMID: 34065512

LABORATORY OF

MECHANISMS OF GERM CELL DEVELOPMENT

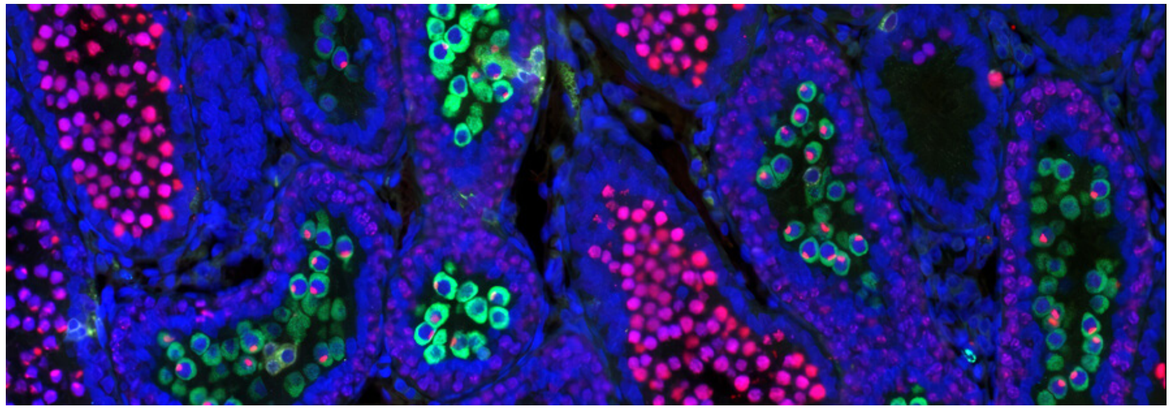
Fertility, meiosis, spermatogenesis, oogenesis, PRDM9, reproductive age

Zdeněk Trachtulec



In the picture: 1. Trachtulec Zdeněk | 2. Tůmová Lucie | 3. Pírková Daniela | 4. Mihola Ondřej

Our society faces new challenges, including the increased age of parents and decreasing sperm quality and quantity, which need to be addressed by the fertility research. Mammalian fertility depends on meiotic recombination (the repair of self-inflicted DNA breaks to crossovers). The sites of recombination hotspots (RHS) are determined by DNA-binding meiotic histone-3-methyltransferase PRDM9. Genetic inactivation of *Prdm9* causes complete meiotic arrest in both sexes of the C57BL/6J (B6) mouse; the reason for the arrest and subsequent sterility has been thought to be the relocation of RHS to promoters. However, dogs or birds carry RHS in promoters and yet do not require PRDM9 for fertility. To resolve this discrepancy, we prepared additional *Prdm9*-deficient rodents. In contrast to the B6 mouse, some mouse and rat males lacking PRDM9 were able to produce sperm and some even offspring, despite RHS in promoters. We predicted that these males with rescued fertility could have more efficient DNA repair of relocated RHS. Indeed, we found a positive correlation between the number of crossovers and sperm presence in *Prdm9*-deficient rodent males. In line with this result, we also found that *Prdm9* is important for quantity, motility and structure of sperm in semifertile mouse hybrids. This sperm amount and quality was affected by the level of expression of a proteasomal subunit; the levels of proteasomal subunits are also changed in the sperm of infertile men. We assume that drugs inducing elevated DNA repair targeted into the testes of some human infertility patients could increase crossover rate and lead to functional sperm. Mutations in the human gene encoding PRDM9 have been implicated in Premature Ovarian Insufficiency (POI), a common subfertility disorder reducing reproductive age. Our rat females lacking PRDM9 also display shortened reproductive age. We uncovered a possible mechanism linking POI in mothers with aneuploidy (which includes Down syndrome) in offspring. This link could be non-homologous meiotic synapsis, which causes DNA repair with decreased crossover rate followed by aberrant segregation of chromosomes leading to aneuploid eggs with abnormal chromosome numbers. Our animals thus represent excellent models of the currently important infertility disorders occurring in both men and women.



Cross-sections of 22-day-old rat testes with labelled DNA (blue), a nuclear protein of less advanced sperm precursors (red), and a cytoplasmic protein of more advanced sperm precursors (green) visualized by fluorescence microscopy. Comparing sections from control and mutant testes revealed delayed meiotic progression in mutant males.
Photo: O. Mihola

Selected publications:

1. Kusari F, Mihola O, Schimenti JC, Trachtulec Z*: Meiotic epigenetic factor PRDM9 impacts sperm quality of hybrid mice. Reproduction 2020 160(1): 53-64.
2. Mihola O*, Kobets T, Krivankova K, Linhartova E, Gasic S, Schimenti JC, Trachtulec Z: Copy-number variation introduced by long transgenes compromises mouse male fertility independently of pachytene checkpoints. Chromosoma 2020 129(1): 69-82.
3. Mihola O, Landa V, Pratto F, Brick K, Kobets T, Kusari F, Gasic S, Smagulova F, Grey C, Flachs P, Gergelits V, Tresnak K, Silhavy J, Mlejnek P, Camerini-Otero RD, Pravenec M, Petukhova GV, Trachtulec Z*: Rat PRDM9 shapes recombination landscapes, duration of meiosis, gametogenesis, and age of fertility. BMC Biol 2021 19(1): 86.
4. Gasic S, Mihola O, Trachtulec Z*: *Prdm9* deficiency of rat oocytes causes synapsis among non-homologous chromosomes and aneuploidy. Mamm Genome 2022 33(4): 590-605.

NATIONAL RESEARCH INFRASTRUCTURES AND RESEARCH CENTRE

NATIONAL INFRASTRUCTURE FOR

CHEMICAL BIOLOGY

Petr Bartůněk

In the picture: 1. Popr Martin | 2. Pavlová Viola | 3. Stillerová Vendula | 4. Vonková Ivana | 5. Králová Jarmila | 6. Zbončáková Adriána | 7. Lisová Michaela | 8. Martinková Olga | 9. Bražinová Jana | 10. Franke Kidorová Dita | 11. Bartůněk Petr | 12. Voršilák Milan | 13. Müller Tomáš | 14. Kaňovský Aleš | 15. Bojić Milan | 16. Škuta Ctibor | 17. Dušek Vladimír

CZ-OPENSCREEN operates the most advanced research infrastructure for basic and applied research in the fields of chemical biology and genetics in the Czech Republic and provides open access to its external users. It supports this new interdisciplinary research by bridging traditional natural sciences such as cell biology, molecular and structural biology, biochemistry, organic chemistry and chem/bioinformatics. The main mission of CZ-OPENSCREEN is to identify new molecular probes and to develop new tools for the research of chemical compounds as candidates for the development of new potential therapeutics. Unlike commercial platforms, CZ-OPENSCREEN also focuses on non-validated molecular targets, signalling pathways and neglected diseases. To those users from the biological and chemical community, CZ-OPENSCREEN offers standard biological and biochemical assays, consultancy and development of new assays, high-throughput screening (HTS), profiling of chemical compounds on a panel of cell lines and medicinal chemistry optimization of newly identified biologically active compounds. CZ-OPENSCREEN is systematically building a library of both commercial chemical compounds as well as compounds synthesized in the Czech Republic. It provides access to this unique library to external users. An integral part of the services is cheminformatics support, such as data analysis and storage, the development of new analytical tools and database systems. CZ-OPENSCREEN is equipped with state-of-the-art technologies for high-throughput screening of chemical compounds such as integrated robotic HTS stations, robotic stations for performing automated microscopic analyses and label-free technology including mass spectrometry, integrated robotics systems for compound storage and sample preparation. The long-term international collaboration of CZ-OPENSCREEN with other European partner institutions has contributed to the establishment of the European Research Infrastructure Consortium EU-OPENSCREEN ERIC (European Infrastructure of Open Screening Platforms for Chemical Biology). The Czech Republic is among the founding Member States. CZ-OPENSCREEN is its national node and besides other activities, it hosts the European Chemical Biology Database (ECBD), where all the data generated by EU-OPENSCREEN ERIC partner sites are stored.

The acoustic compound transfer unit is part of the High-throughput screening system.

CZECH BIOIMAGING MICROSCOPY CENTRE

Pavel Hozák

In the picture: 1. Pišlová Lenka | 2. Hozák Pavel | 3. Wernerová Martina | 4. Klimešová Daniela

Czech-BioImaging is a national infrastructure for biological and medical Imaging. The research infrastructure is distributed and consists of 16 imaging facilities at 10 organizations in Prague, Vestec, Brno, České Budějovice and Olomouc. From 2016, Czech-BioImaging provides users with open access to a wide range of imaging methods and expertise e.g. advanced light microscopy, fluorescence microscopy, super-resolution microscopy, cryo/electron microscopy, sample preparation, magnetic resonance imaging, tissue and organ imaging, magnetic particle imaging and image analysis. Through 3 national nodes, the infrastructure is a part of the pan-European infrastructure for biological and medical imaging Euro-BioImaging ERIC.

Czech-BioImaging covers all levels of biomedical imaging – from imaging of biomolecules and their interactions, structure and processes in cells and tissues, to imaging of organs and whole organisms, both in healthy and pathological condition. Its strength is the unique combination of top available equipment, extensive expertise in imaging, emphasis on education and tight collaboration with commercial sector. Added value is its own methodological research, especially in the field of development and implementation of new imaging methods at the core facilities.

The main aims of Czech-BioImaging are:

- to enable permanent access to cutting-edge imaging technologies and expertise
- to increase awareness of progressive biological and medical imaging methods
- to support mutual cooperation of scientists and stimulate inter/national sharing of knowledge

Czech-BioImaging is included in the Roadmap of Large Infrastructures for Research, Experimental Development and Innovation of the Czech Republic for the years 2016 - 2022.

Czech-BioImaging is supported from the programme for large research infrastructures of the Ministry of Education, Youth and Sports.

The Microscopy Centre coordinates and provides logistic services to the large research infrastructures Czech-BioImaging and Euro-BioImaging. The Centre mainly serves as the central hub of the Czech-BioImaging infrastructure and as the coordinator of the Prague Node of the Euro-BioImaging ERIC consortium.

With the affiliated microscopy facilities, the Centre offers users open access to a wide range of state-of-the-art light and electron microscopy instruments and techniques, consultation services, sample preparation, data acquisition and analysis, image processing tools, and interpretation of the obtained results.

The Centre with the light microscopy and electron microscopy core facilities organize training workshops in the field of microscopy. The Centre cooperates on a long-term basis with other members of national microscopy activities; the Czechoslovak Microscopy Society and microscope manufacturers with the focus on new microscopy methods and innovation of the current microscopy instruments.

The Microscopy Centre is supported by the Ministry of Education, Youth and Sports.

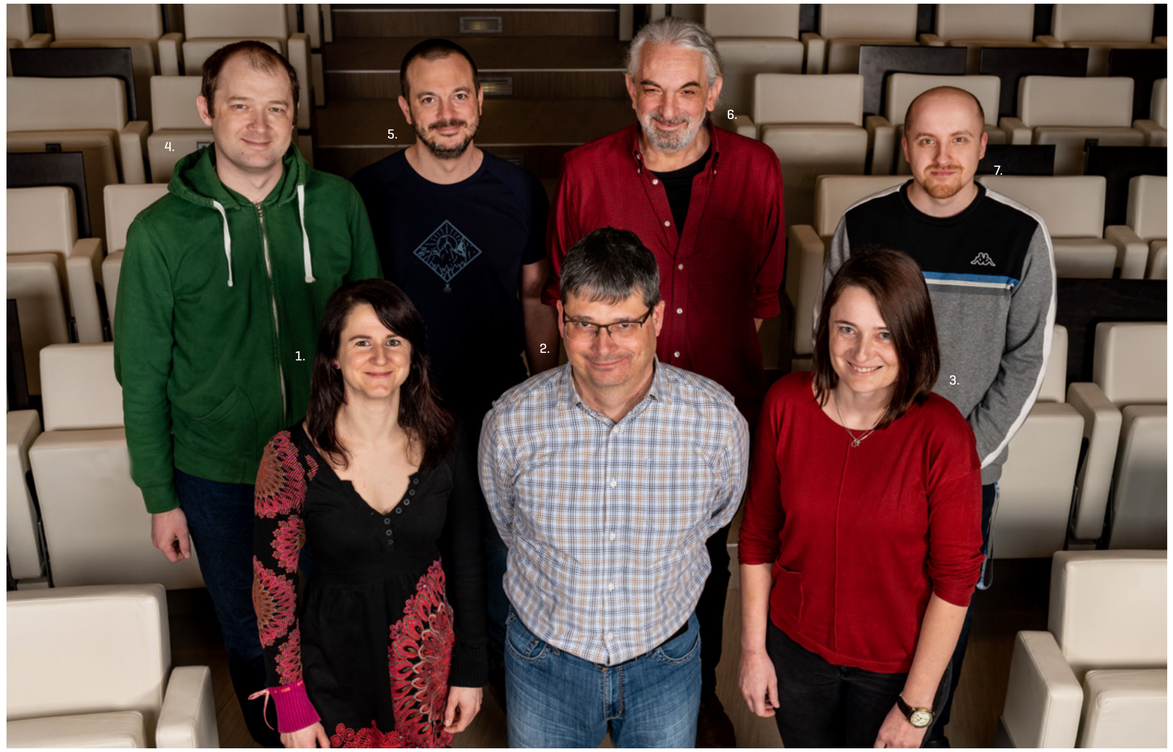
LIGHT MICROSCOPY

Ondrej Horváth

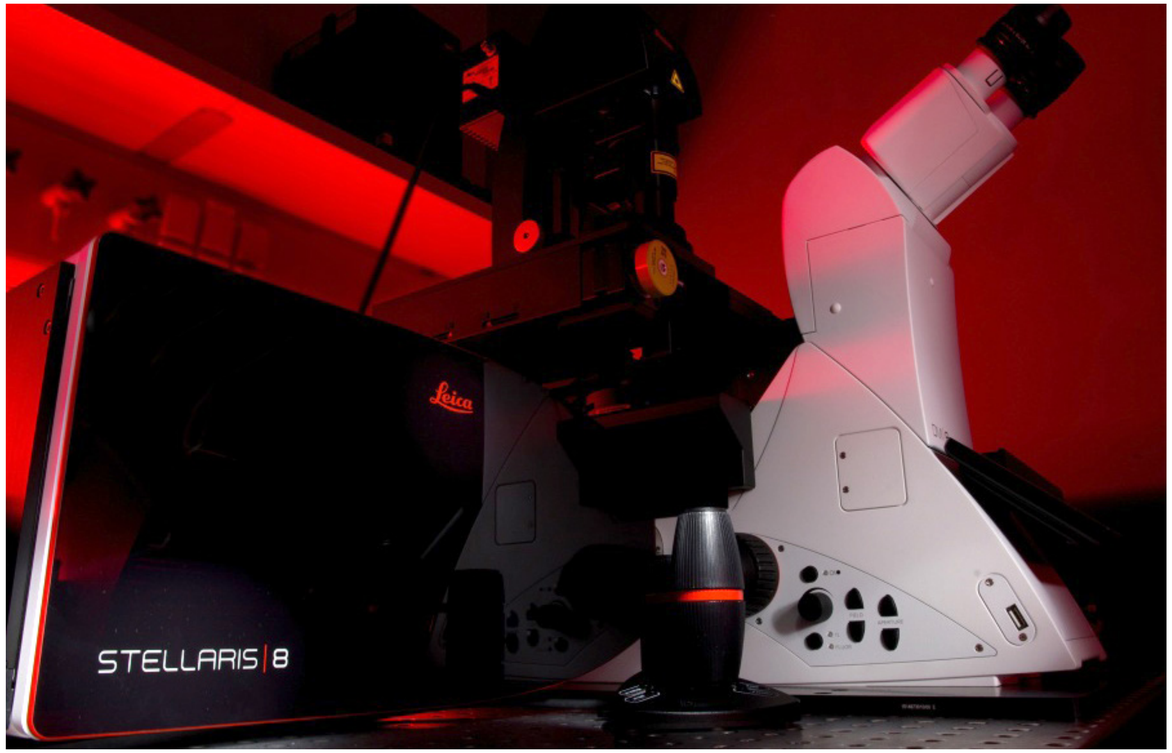
Light Microscopy Core Facility of the Institute of Molecular Genetics of the Czech Academy of Sciences (LMCF IMG) is a state-of-the-art research facility equipped with a wide range of high-end microscopy technologies and image processing tools. LMCF IMG is located in the IMG main building in the biomedical campus Krč of the Czech Academy of Sciences. Most of the instrumentation in the facility is available on the self-service basis, for trained users. User access to the facility is supported via "Czech-Bioimaging open access" program both for in-house and external scientists.

The available technologies range from simple widefield and confocal fluorescence microscopes to more advanced systems such as light-sheet, confocal spinning disc and superresolution techniques SMLM, SIM, SRRF and STED. We put the emphasis on training of the users, starting with introductory courses for the beginners and continuing to specialized trainings for more experienced microscopists. We also offer personalized help with project planning, sample preparation, instrument usage and optimization of the imaging. Moreover, we provide expertise on final image processing and analysis, including image registration, routine deconvolution, tracking, image segmentation, analysis of photo-kinetic experiments, mathematical modeling and simulations of biological processes etc. Finally, our team organize intensive advanced courses in light microscopy and image processing and analysis techniques.

LMCF IMG is involved in the national infrastructure for biological and medical imaging – Czech-Bioimaging. Along with the imaging facilities of the Institute of Physiology of the Czech Academy of Sciences, the Charles University in Prague (BIOCEV), Biology Centre of the Czech Academy of Sciences and the Institute of Experimental Botany of the Czech Academy of Sciences, we constitute the Prague node of the Czech-Bioimaging. In 2020, the node became a member of the pan-European imaging infrastructure, Euro-Bioimaging.



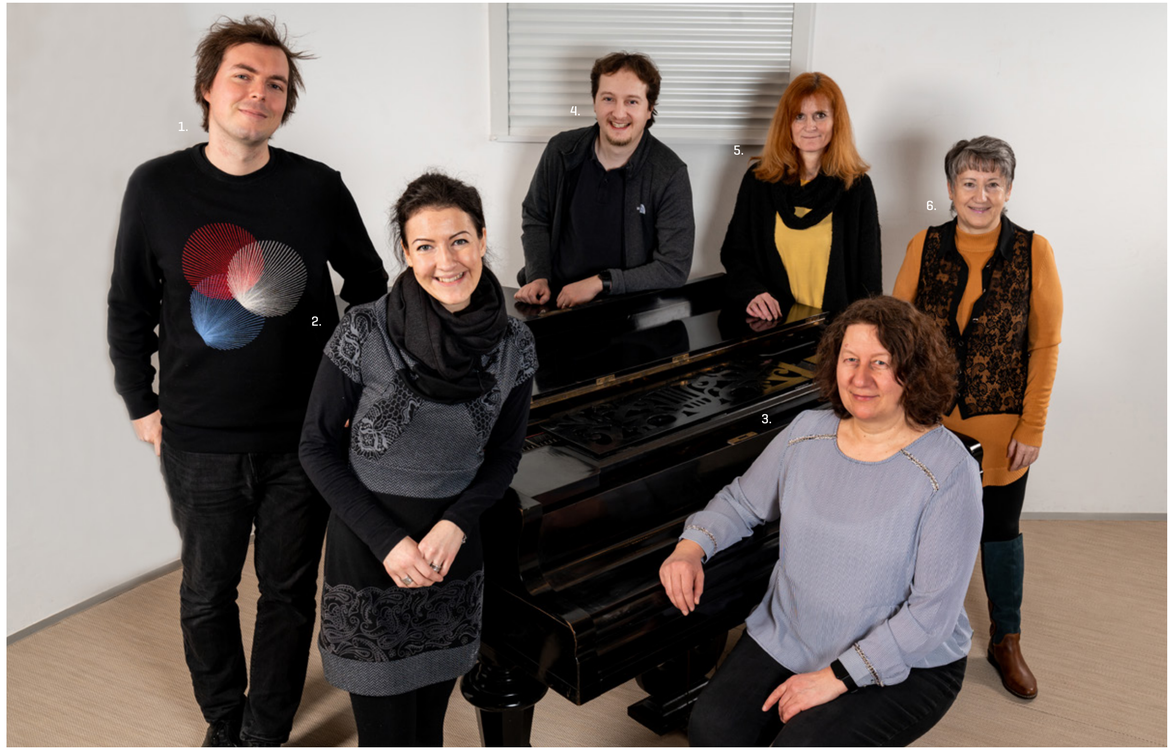
In the picture: 1. Blažíková Michaela | 2. Čapek Martin | 3. Chmelová Helena | 4. Valečka Jan | 5. Novotný Ivan | 6. Horváth Ondrej | 7. Černý Jiří



Leica STELLARIS 8 FALCON with the LIGHTNING adaptive deconvolution

ELECTRON MICROSCOPY

Vlada Filimonenko



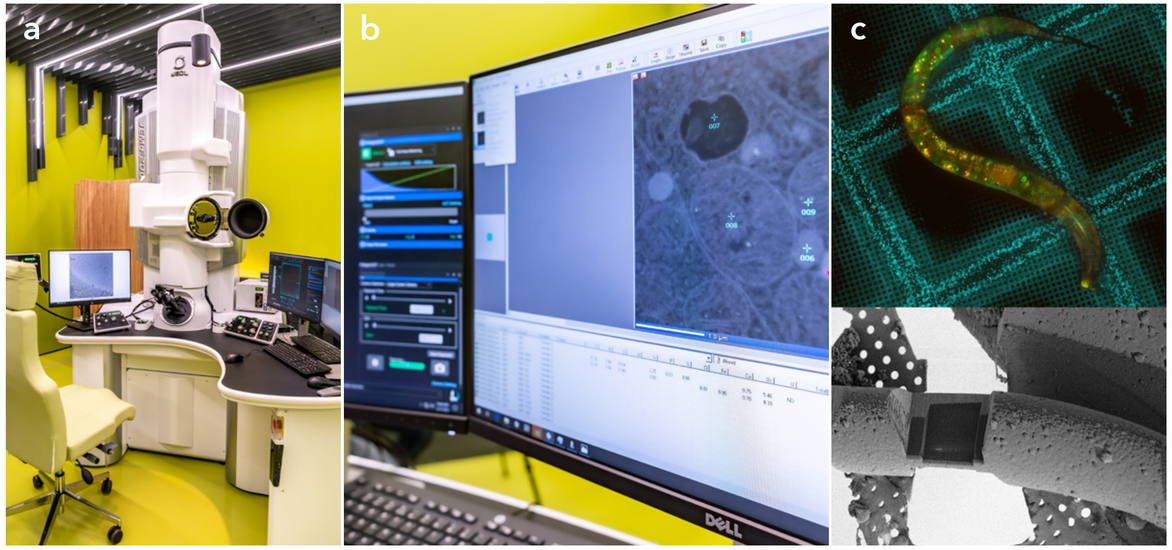
In the picture: 1. Vlčák Erik | 2. Raabová Helena | 3. Filimonenko Vláda | 4. Pinkas Dominik | 5. Pišlová Lenka | 6. Nováková Ivana

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, cryofixation using high-pressure freezing technique, freeze-substitution, plunge-freezing, cryosectioning, and immunolabeling, including simultaneous detection of multiple targets by our self-developed methods.

The core facility is equipped with two transmission electron microscopes (TEM) installed in November 2019 – a standard instrument for routine observation and an advanced 200 kV instrument providing the possibility of high-resolution TEM, STEM, 3D electron tomography, cryo-electron microscopy and EDS elemental analysis and mapping. For sample preparation, a high-pressure freezing machine, two automatic freeze-substitution machines, freeze-fracture and replica making device, cryo-ultramicrotomes, Leica EM GP2 for automated plunge-freezing, as well as additional wet lab equipment are available. Specialized light microscope with LN2-cooled stage provides possibility for correlative light and electron microcopy workflows in ambient and cryo conditions.

Our team has a long expertise in the development and optimization of sample preparation techniques for electron microscopy. Recently, we developed a free online tool for quantification of immunolabeling in electron microscopy (https://pattern.img.cas.cz/). In the frame of tripartite collaboration with TESCAN Brno and Leica Microsystems companies we established and optimized multiple workflows based on FIB-milled frozen hydrated lamella, using the TESCAN Aber Cryo FIB-SEM installed at our facility.

We provide open access for our technologies and expertise via Czech BioImaging and Euro BioImaging infrastructures, being a part of the IMG Czech-BioImaging node and Prague Euro-BioImaging node. As the spectrum of approaches and workflows in electron microscopy is very wide, we help the users to select an appropriate technique and to plan the whole experiment. The sample preparation and image acquisition can be done fully by facility staff or we can provide sufficient training and initial support for independent use of the technologies and equipment. We organize a yearly one-week practical course of transmission electron microscopy in life sciences for beginners and intermediate users.



(a) Transmission electron microscope JEM-F200 „F2" operated at 200 kV enables various modes of imaging and sample analysis: TEM, STEM, cryo-TEM/STEM, volume reconstruction by electron tomography, and elemental mapping by EDS. (b) EDS spectra collected at specified points of the biological sample contain information about its chemical composition. (c) A model worm C. elegans expressing two fluorescently-tagged proteins was vitrified and observed in fluorescent microscope under cryo conditions (upper image) and the region of interest was thinned down in cryoFIB-SEM (bottom image) for subsequent electron tomography.

Czech Centre for Phenogenomics

CZECH CENTRE FOR PHENOGENOMICS

Radislav Sedláček

In the picture: 1. Šroubková Jarmila | 2. Šafránková Jana | 3. Morská Markéta | 4. Červená Lenka | 5. Sedláček Radislav

The Czech Centre for Phenogenomics (CCP) is a non-distributed biomedical large research infrastructure and provides a unique comprehensive preclinical research service for users from the Czech Republic as well as the international ones. CCP has gained a worldwide reputation for the quality of its service and publication results, and has a strong position in international consortia, the IMPC (International Mouse Phenotyping Consortium) and the European INFRAFRONTIER. Thanks to its membership in INFRAFRONTIER, CCP has licenses for gene editing with CRISPR technology and is the only centre in the country allowed to officially create and distribute models prepared with this technology. A key facility of CCP is the Phenotyping Module, which performs standardized high-throughput analysis of transgenic animal models capturing nearly 1000 unique parameters for each gene analyzed and the effect of its mutation in the organism or in the development and treatment of disease. In recent years, CCP has newly built a platform for PDX models to investigate cancer and human cancer therapies using mouse models and state-of-the-art BSL-3 laboratory to investigate dangerous infections (Covid-19, hepatitis, etc.) in mouse models (these laboratories are virtually unavailable as a service in the country).

CCP has established a comprehensive phenotyping pipeline, allowing detailed and standardized description of the impact of a gene of interest in the organism. CCP can investigate all main physiological systems and reveal how and where the gene functions. All procedures and technologies are standardized, which improves results reproducibility. Successful development of CCP proves that the centre has the potential to be at the forefront of health research and contribute to new cutting-edge developments.

CCP is divided into three major modules: i) Transgenic and Archiving Module (TAM), where new animal models are generated, ii) Animal Facility Module (AFM), responsible for housing and breeding, and iii) Phenotyping Module (PM), providing standardized and large-scale phenotyping, user-tailored services as well as the preclinical research.

CCP not only develops new technologies for genome manipulation and characterization of physiological functions, but also provides services in preclinical research, thus contributing to the development of new therapeutics.

Czech Centre for Phenogenomics

TRANSGENIC AND ARCHIVING MODULE [TAM]

Radislav Sedláček

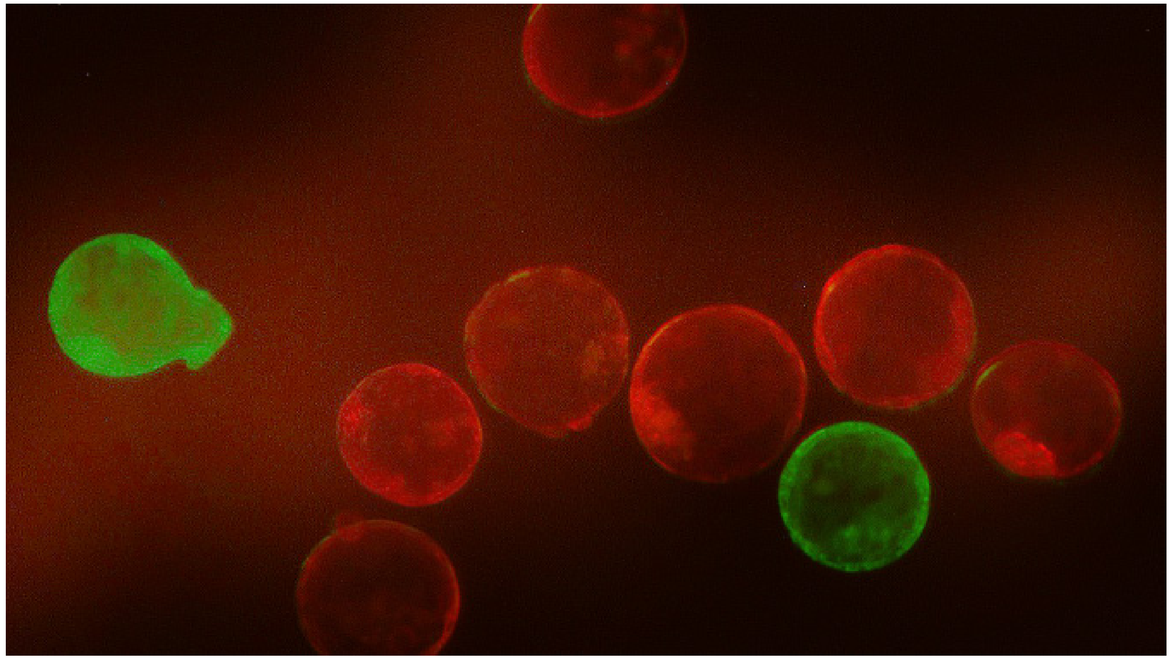
In the picture: 1. Hořejšová Sandra | 2. Michaliková Csilla | 3. Sedláček Radislav | 4. Kopkanová Jana | 5. Nickl Petr | 6. Vikhrova Elena | 7. Šolcová Katarzyna Daria | 8. Josková Markéta | 9. Zelená Kateřina | 10. Tkadlecová Anna | 11. Krupková Michaela

Transgenic and archiving module (TAM) has established a comprehensive technology portfolio for rodent model generation fully comparable with any world-class facility in this specific area and is thus a key part of Czech Centre for Phenogenomics, responsible for generation of novel genetically modified mice and rats using state-of-the-art technologies. TAM generates rodent models in-line with IMPC and INFRAFRONTIER standards and guidelines and consists of the Genome Engineering & Model Generation and the Genotyping and Breeding, Cryopreservation & EMMA/INFRAFRONTIER units. TAM provides complete service, from the initial gene-targeting design, generation of tools and transgenic rodent models to the genotyping and breeding of desired animal models.

The most used genetic background in CCP is C57Bl/6N, but we can generate models on various backgrounds. Although most newly generated mutant rodents are "knock-out" or "knock-in" models based on CRISPR/Cas9 targeting tools and zygote electroporation, transgenic models e.g., via pronuclear injection (PNI) of plasmid or BAC DNA can be also generated. Founder and G1 mice are analyzed to confirm germ line transmission (GLT). The successfully produced mouse/rat lines are cryopreserved (embryo or sperm cryopreservation). Furthermore, we offer mice production with the ES targeting technologies. Routinely we produce models from targeted embryonic stem cells originating from EUCOMM and KOMP repositories. Majority of modifications in these ES cell lines are so called "knockout-first" alleles that represent a LoxP-flanked critical exon with LacZ reporter element.

In cooperation with animal facility of CCP we provide consultation, assistance services, and information on the design and use of genetically modified transgenic mice. We also assist in animal rederivation (cleaning of the rodent line), reanimation (creating of the line from frozen embryos or sperms) as well as models import and export using cryopreserves sperm and embryos.

TAM provides services to a broad national and international scientific community. As a member of INFRAFRONTIER, we are contributing with mice generation to the IMPC project that aims to knockout all the mammalian genes. We also represent a Czech node of EMMA (European Mouse Mutant Archive), a non-profit repository for the collection, archiving (via cryopreservation) and distribution of relevant mutant mouse strains essential for basic biomedical research.



Testing the conversion from red fluorescent protein to green fluorescent protein in mouse mT/mG blastocysts.

Czech Centre for Phenogenomics

ANIMAL FACILITY KRČ

Jan Honetschläger

In the picture: 1. Oros Tetyana | 2. Polevičová Lenka | 3. Doubravová Romana | 4. Siváková Ludmila | 5. Vorlová Daniela | 6. Fechka Tetiana | 7. Rynekrová Markéta | 8. Adamec Tomáš | 9. Vávrová Gabriela | 10. Bakešová Zuzana | 11. Králová Alena | 12. Kubová Veronika | 13. Zukalová Jitka | 14. Novotná Monika | 15. Schůtová Pavla | 16. Svobodová Michaela | 17. Kratochvílová Daniela | 18. Dygryn Stanislav | 19. Koláčná Klára | 20. Šimanová Martina | 21. Kádrle Matěj | 22. Kupčíková Markéta | 23. Gašpierik Tomáš | 24. Herodes Martin | 25. Smetana Miroslav | 26 Pelikánová Anežka | 27. Honetschläger Jan

The IMG animal facility on the Krč campus provides superior animal housing and breeding standards, including sufficient experimental space. The facility is fully accredited for breeding common and genetically altered animal strains. The current total capacity is more than 9,000 cages and is split into different sections: conventional breeding, quarantine for imported animals, and „specified pathogen-free" breeding barrier facility operated under the FELASA guidelines. The barrier facilities are equipped with steam sterilisers, vaporised hydrogen peroxide chambers, pass-through boxes, and individually ventilated cages to enhance the bioexclusion. The facility delivers its services mainly to IMG users and other cooperating institutes.

Czech Centre for Phenogenomics

ANIMAL FACILITY MODULE (AFM) VESTEC

Libor Kopkan

In the picture: 1. Kučerová Daniela | 2. Kukačková Julie | 3. Pilařová Anna | 4. Glosová Svatava | 5. Závodská Zdeňka | 6. Kopkan Libor | 7. Dřízhalová Martina | 8. Šustrová Romana | 9. Agócsová Bernadett | 10. Grassingerová Petra | 11. Babanská Alena | 12. Dufková Lucie | 13. Kameníková Pavla | 14. Olah Eduard | 15. Kallischková Marie | 16. Vaníková Jana | 17. Shevchuk Lyudmila | 18. Markova Ekaterina | 19. Nehrych Mariia | 20. Hradil Sebastien | 21. Hradilová Marie | 22. Čermák Antonín | 23. Matoušková Jana | 24. Roth Jiří | 25. Kratochvílová Alena | 26. Bursa Michal | 27. Froňková Jiřina | 28. Šnajdrová Pavla | 29. Černá Alena | 30. Tačnerová Zuzana | 31. Humlová Elena

The Animal Facility Module (AFM) of the Czech Centre for Phenogenomics is based on the latest advances in housing, breeding and care of laboratory mice and rats. We have built one of the most progressive animal facilities regarding logistics, versatility and demand for animal health and welfare.

The CCP animal facility contains five individual, fully separated breeding, and experimental barrier areas as well as an autonomous quarantine area. Each barrier includes modern devices such as large volume steam sterilizers and H_2O_2 chambers, air- or wet-shower entry areas, pass through biosafety cabinets, modern and eco-friendly HVAC technology.

All these important devices help us to keep a controlled SPF (specific pathogen-free) environment in the barriers. All animals are housed in individually ventilated cages (IVC) or digitally ventilated cages (DVC). This state-of-the-art-technology significantly improves the animal welfare level and animal facility efficiency as well as it allows the detection of anomalous behavior (sick or wounded animals), reduce animal stress and provide continuous information about animal activity. To facilitate all animal care procedures, AFM employs latest semi-automated washing technology including a tunnel cage washer, bottle washer, rack washer, and waste disposal and bedding dispensing vacuum system to increase the in-house biosecurity.

Conception of buildings and animal facility management is in accordance with the highest world standards for laboratory animals and in compliance with EU legislation. AFM is regularly inspected by appropriate authorities.

Picture of the IVC housing system for laboratory mice in the breeding barrier.

Czech Centre for Phenogenomics

THE PHENOTYPING MODULE (PM)

Radislav Sedláček

In the picture: 1. Magalhães Novais Silvia Carina | 2. Nichtová Zuzana | 3. Sedláček Radislav | 4. Špoutil František | 5. Novosadová Vendula | 6. Procházka Jan | 7. Holá Hana | 8. Mastrangeli Antonia | 9. Machancoses Hernández Francisco | 10. Procházková Michaela | 11. Richtárechová Pavlína | 12. Štefancová Eva | 13. Martínková Veronika | 14. Buková Ivana | 15. Kubik-Zahorodna Agniezska | 16. Pajuelo Reguera David | 17. Zudová Dagmar | 18. Długošová Sylvie | 19. Fedosieieva Olha | 20. Juhász Attila | 21. Symkina Viktoriia | 22. Křížová Kamila | 23. Dowling Laura | 24. Vičíková Kristina | 25. Balounová Jana | 26. Šímová Michaela | 27. de Guia Roldan Medina | 28. Lindovský Jiří | 29. Macek Petr | 30. Raishbrook Miles | 31. Suchanová Šárka | 32. Pálková Marcela | 33. Valentová Anna | 34. Bohinskyi Pavlo | 35. Biryukova Evgeniya | 36. Klíma Kryštof | 37. Dohnalová Klára | 38. Kučera Lukáš | 39. Madureira Trufen Carlos Eduardo

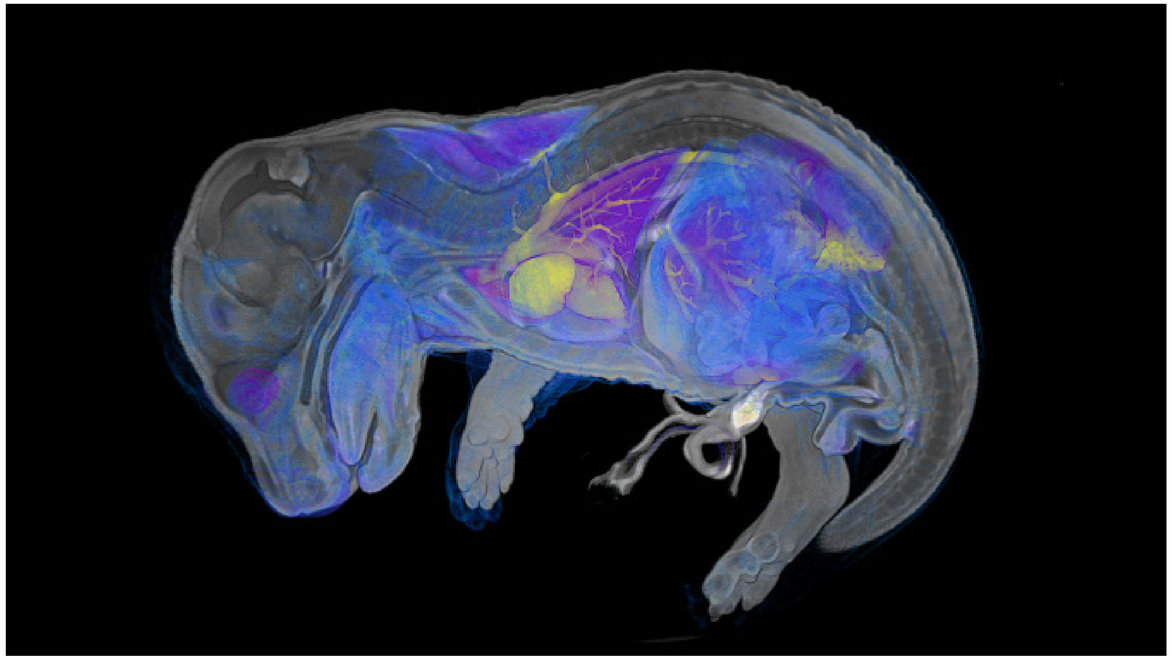
The Phenotyping Module (PM) is an integral part of the national large research infrastructure Czech Centre for Phenogenomics (CCP). The Phenotyping Module has been operating since 2016 and comprises several specialized laboratories and a compendium of technologies and expertise for investigating the major physiological systems of the body.

The full list of technologies together with a list of equipment can be found at: http://www.phenogenomics.cz/phenotyping/. The technologies accompanying specific services are grouped into units: 1/ histopathology, 2/ immunology, 3/ cardio-vascular, 4/ metabolism, 5/ biochemistry, 6/ metabolomics, 7/ bioimaging, 8/ embryology, 9/ lung function, 10/ neurobehavioral, 11/ hearing, 12/ vision, 13/ PDX/ cancer models, 14/ bioinformatics, and a new preclinical R&D unit. All these CCP units together with their specific sets of technologies create a unique collection of expertise in a single location. In total, the available technologies enable collecting more than 700 parameters for each phenotyped animal. Moreover, the portfolio of services and parameters is continually developing.

The units of Metabolism and Clinical Biochemistry build together the Metabolomics platform and intensively interact with histopathology and the newly built MALDI-Imaging and Bioinformatics unit.

The Phenotyping Module is also a key module regarding service for users and contract research, especially in the preclinical development. The latest addition of the PM is the BSL3-barier, the most advanced facility in the Czech Republic, to study infection diseases such as Covid-19 or hepatitis using rodent models.

The specialized modules of CCP provide open access-based services to groups and institutions from the entire Czech Republic irrespective of whether they are from the Czech Academy of Sciences or universities. Moreover, CCP also provides open access to international customers from Europe and worldwide, and the number of all these customers is steadily growing.



MicroCT imaging of mouse embryo, the contrast is enhanced by pseudocolors to highlight internal organs.

ELIXIR CZ

Jan Pačes

The Czech ELIXIR Node (part of ELIXIR-EU) represented by the Institute of Molecular Genetics and Institute of Chemistry and Biochemistry of the Czech Academy of Sciences is a national hub for bioinformatics, comprising an advanced computational environment, dedicated data collections and unique tools provided for the life science community. The Node has been created and operated as a distributed infrastructure, currently comprising fourteen organizations. Partner institutions provide tools and databases in an open access regime to Czech scientists and the international research community.

There are three strong areas in which the Node provides a connection between the national and pan-European research structures:

1. The most advanced and developed area belongs to the Structural Bioinformatics field focusing mainly on services and development of specific tools. This field is very tightly connected with Czech IT research infrastructure partners – namely CESNET and CERIT SC – providing not only computational and storage capacity, but also know-how in the networking and use of heterogeneous resources.
2. The second largest area is represented by curated databases in two major areas – specific collections of genomic data on microorganisms and plants and cheminformatics databases, which also serve as an interconnecting element between other biomedical infrastructure projects such as EU-OPENSCREEN and EATRIS.
3. Last but not least, the IT dedicated solutions for the ELIXIR infrastructure are developed by the Node IT partners, and their involvement in other ELIXIR CZ projects represents a common platform for unification and efficiency of the whole process. The Node also serves as a coordination platform of national bioinformatics activities and is responsible for data uniformity, ELIXIR-standard application and for world-scale accessibility in the whole research space within the Czech Republic

RESEARCH SERVICES

RESEARCH SERVICES

CHICKEN FACILITY KOLEČ

Martina Minariková

The chicken breeding facility is located in the village Koleč, north of Prague, about 45 km from IMG. It includes mainly rears genetically defined inbred, congenic and outbred chicken lines. The breeding facility produces eggs, embryos and chickens for several research groups focusing on the chicken model. Chickens are mostly used mainly for virological or immunological projects, currently for the studying of avian leukosis viruses, which, among other things, serves as a model for the study of other retroviruses, such as human immunodeficiency virus.

In the picture: 1. Thunová Kamila | 2 Pudilová Michaela | 3. Hrodková Iva | 4. Burgerová Anna | 5. Pokorná Iveta | 6. Brejníková Petra | 7. Eisensteinová Alena | 8. Dvořáková Jitka | 9. Minariková Martina

Preparatory and washing room in the new breeding hall for parent poultry breeding.

IONIZING RADIATION SOURCES

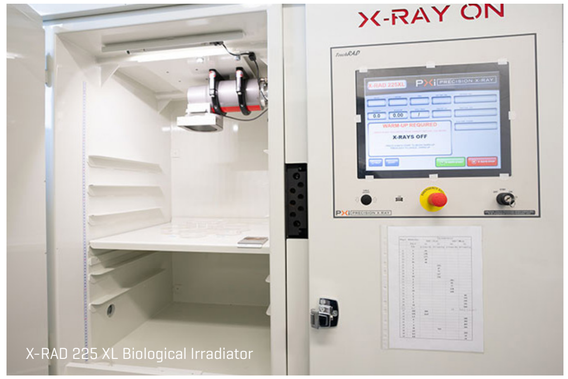
HANDLING CORE FACILITY

Stanislav Pavelka

The facility has provided services already for two decades to support various needs of all users of ionizing radiation sources at the Institute. Members of research groups at IMG routinely use two very different types of ionizing radiation sources, demanding various methodological and instrumentation background provided by the facility: A. Unsealed radionuclide sources, mainly in the form of radioactively labelled organic compounds, for biochemical and pharmacological studies of metabolism; B. X-ray generators in the form of compact cabinet biological irradiators, for regulated X-ray irradiation of small laboratory animals (mice) and samples of cultured cells.

For users performing routinely regulated X-ray irradiation of samples of biological materials, the core facility is equipped with very comfortable, compact cabinet X-RAD 225 XL Biological Irradiator. Trained personnel – users qualified by the facility leader may use this instrument themselves (after the previous reservation in the Calpendo system) and without time limitations, under the specified conditions (see document Rules and Pricelist at the web address: https://www.img.cas.cz/group/x-ray-irradiation-facility/)

For the Director of the Institute, the facility leader acts as a link between the Institute and The State Office for Nuclear Safety (SÚJB), which demands permanent, annual updating of documentation regarding the conditions and working places within the Institute (in compliance with the Atomic Act no. 263/2016 Coll.).



X-RAD 225 XL Biological Irradiator

CRYOBANK BIOCEV

Jana Kopkanová

The Cryobank in Vestec became operational in March 2016. The cryobank is intended for long-term storage of samples and is divided into two parts. The first part is situated in the main building of the BIOCEV Centre and is mainly intended for storage of cell lines and hybridomas.
The second part is located in building SO-002 as a part of the Transgenic and Archiving Module IMG CAS. This part of the cryobank mainly serves for preservation of mouse sperm and mouse embryos in liquid nitrogen or its vapours.

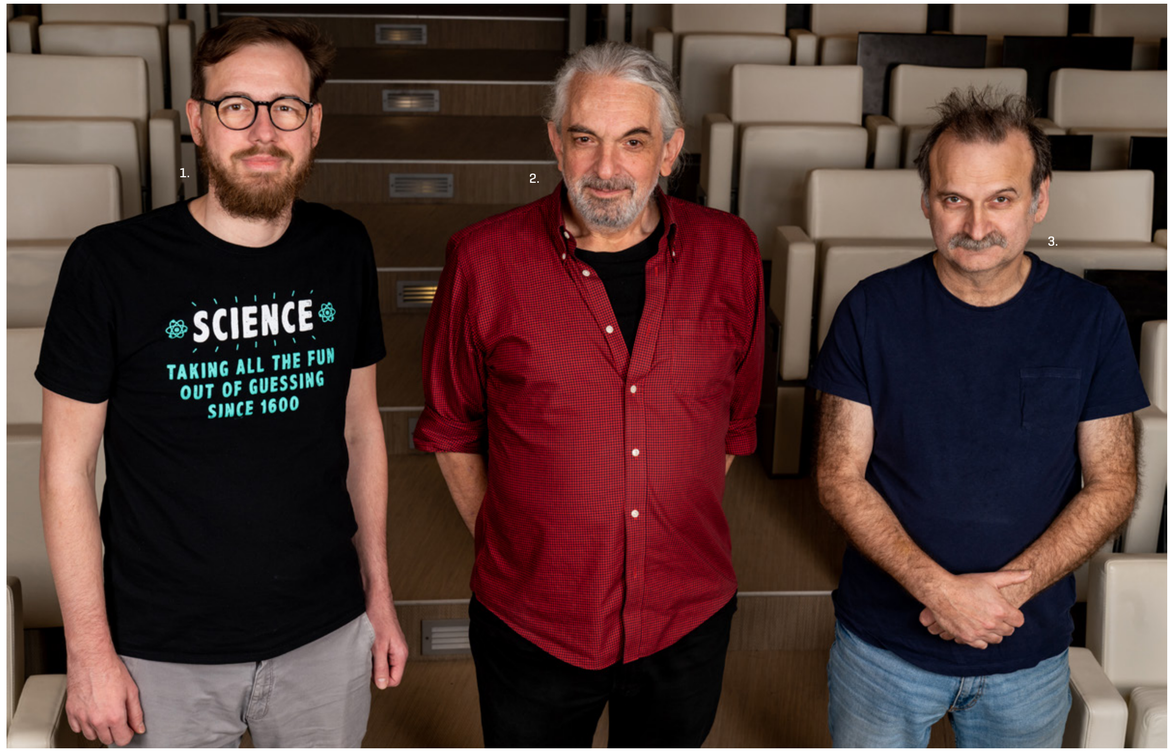
The storage containers (LABS40K, LABS80K – Taylor-Wharton and 24K) are connected to an external reservoir of liquid nitrogen with a capacity of 10,000 litres and are refilled automatically. The cryobank also includes four filling stations providing the possibility to pump liquid nitrogen into both pressure and non-pressure containers. The entire cryobank system is connected to a back-up power supply for the cases of power outage. All operations, diagnostics and checking of the liquid nitrogen level in the storage containers are fully automated and controlled. The parameters (temperature, humidity, O2 concentration) and the safety in both the cryobank and the storage containers themselves are controlled by a monitoring system connected to GSM and a web interface.

The cryobank core facility also administers production of dry ice.


Mobile dewar LS750 Taylor-Wharton used for mouse sperm freezing and goblet system for long storage in LN2.

FLOW CYTOMETRY

Ondrej Horváth



In the picture: 1. Šíma Matyáš | 2. Horváth Ondrej | 3. Cimburek Zdeněk

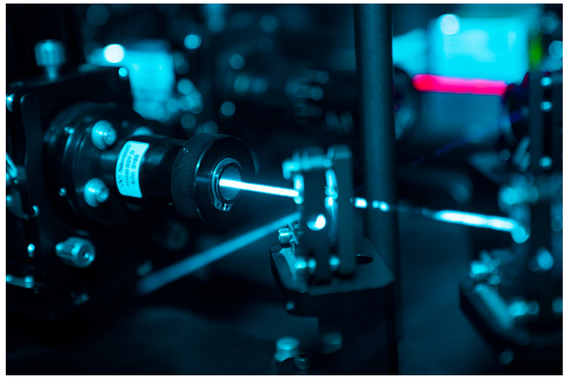
The department provides methodological and instrumentation background for flow cytometry techniques. The facility is equipped with five flow cytometers – two conventional analysers (BD FACSymphony and BD LSRII), one imaging flow cytometer (Amnis® ImageStream®X Mk II) and two sorters (BD Influx and BD FACSAria IIu).

BD FACSymphony, a high-end cytometer and with six solid-state lasers (355, 405, 445, 488, 561 and 640 nm) and 31 fluorescence detectors, is capable to cover most of the flow-cytometry applications. BD LSRII is equipped with four solid-state lasers (405, 488, 561 and 637 nm) and 13 fluorescence detectors. Both analysers are equipped with an HTS loader for high throughput analysis using 96- or 384-well plates.

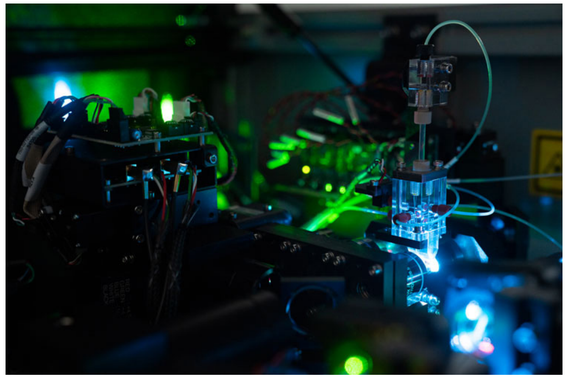
Amnis® ImageStream®X Mk II imaging cytometer has six solid state lasers, four for fluorescence excitation (405, 488, 561 and 642 nm) one for SSC (785 nm) and one for stream calibration. Cytometer is equipped with two cameras, which can acquire images in 12 channels in total. LED diodes serve as the light source for brightfield. Automatic loader allows acquisition of samples directly from 96-well plates.

High-speed jet-in-air cell sorter BD-Influx is equipped with five lasers (355, 405, 488, 561 and 638 nm), 14 fluorescent detectors, small particle option for measuring small particles, and 6-way sorting capability. The BD FACSAria sorter is a cuvette-based high-speed cell sorter with five lasers (405, 445, 488, 561 and 637 nm), 18 fluorescence detectors, and 4-way sorting capability. Both sorters have a cloning deposition unit and they are located inside a biological safety cabinet and fully adapted for sterile sorting.

The facility is also equipped with an AutoMACS Pro (Miltenyi Biotec) magnetic separator for automatic rapid sorting of cells and BC-30 Vet Auto Hematology Analyzer (Mindray) for analysis of mammal animal blood samples. The facility provides access to a powerful analysis tool – FlowJo software. Two working stations with FlowJo are located directly in the facility and 30 FlowJo licences are distributed among the IMG users.



BD LSR II Flow Cytometer



Amnis ImageStreamX Mk II

MEDIA AND GLASS WASHING

Hana Marxová

The facility is responsible for preparation of media and solutions for tissue culture (from redistilled deionized water and PBS, media such as RPMI, various types of MEM, HBSS, trypsin, to specialized solutions), preparation of bacteriology media and plates, sterilization of solutions and material (vapour sterilization, filtration), distribution of FBS, RPMI Sigma and DMEM Sigma, washing of glass and plastic, decontamination of GMO waste and other hazardous wastes (7000 kg per year) and organization of washing of laboratory clothing (more than 4000 items per year). We also provide distribution of ThermoFisher/Life Technologies products in our Supply Centre.

In the picture: 1. Marxová Hana | 2. Fišerová Jana | 3. Alferiová Lenka | 4. Borovičková Lenka

Autoclaving preparation

Sterile filtration

MONOCLONAL ANTIBODIES AND CRYOBANK

Dobromila Kumpoštová

In the picture: 1. Nezbedová Zuzana | 2. Kumpoštová Dobromila | 3. Borovičková Lenka

Monoclonal Antibodies Facility

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

ELISA testing

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) 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, O2 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

HISTOLOGY LABORATORY

Matyáš Šíma

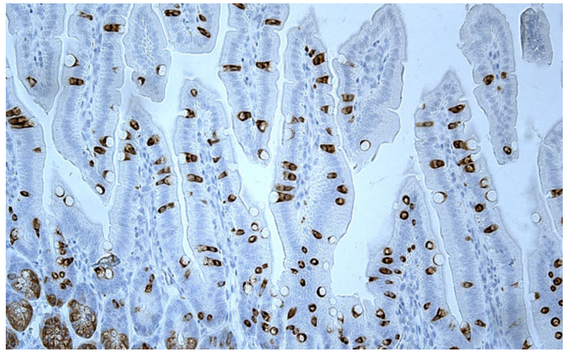
In the picture: 1. Šíma Matyáš | 2. Onhajzer Jakub

Equipment of laboratory allows preparation and sectioning of paraffin blocks, sections deparaffination and antigen retrieval, cryo sectioning and sectioning of fresh and fixed samples on vibratome.

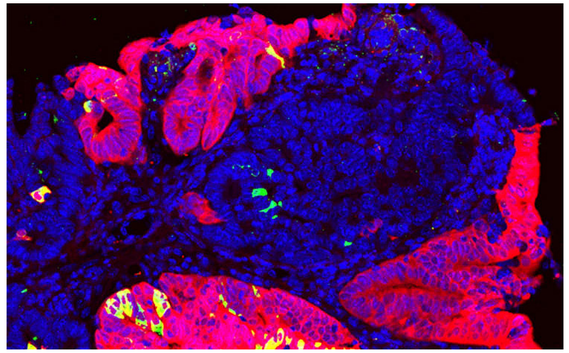
The laboratory equipment consists of a set of Leica devices that include tissue processor HistoCore PEGASUS Plus, HistoCore Arcadia Embedding Center, two fully motorized rotary microtomes RM2255, Cryostat CM1950 and Vibratome VT1200 S.

The tissue processor can process simultaneously up to four hundred samples in two independent retorts. The paraffin-embedding station provides possibilities for creation of different types of wax blocks. The microtomes are supplied with various types of blades for sectioning different tissue types.

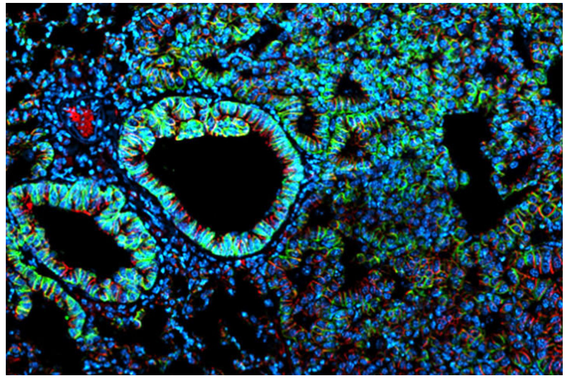
The facility is run as a semi-self-service – tissue dehydration is handled by the staff; all the other steps are carried out by each researcher individually. The facility is open to all academy researchers as well as people from commercial companies, who can use the facility after brief initial training.



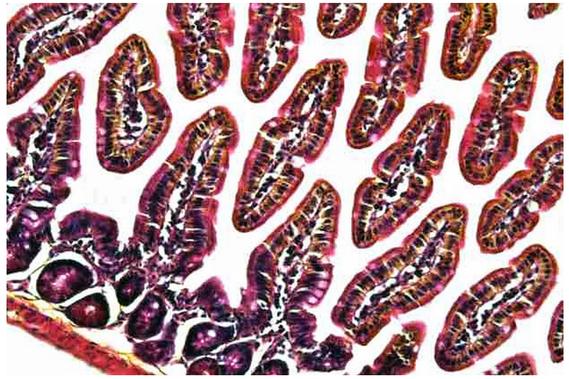
Immunohistochemistry of intestinal secretory cells (brown), cell nuclei contrastained with Hematoxylin (blue-purple)



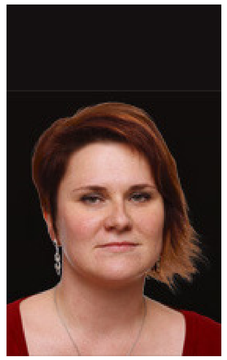
Intestinal tumor, Dapi (Blue; nuclei), ID4 (Green; secretory cells) and Trop2 (Red; tumor cells)



Murine lungs with tumor, Dapi (Blue; Nuclei), EpCam (Green; Epithelial cells) and PCNA (Red; Proliferating cells)



Lendrum straining of the mouse intestine



PROTEOMICS SERVICE LABORATORY

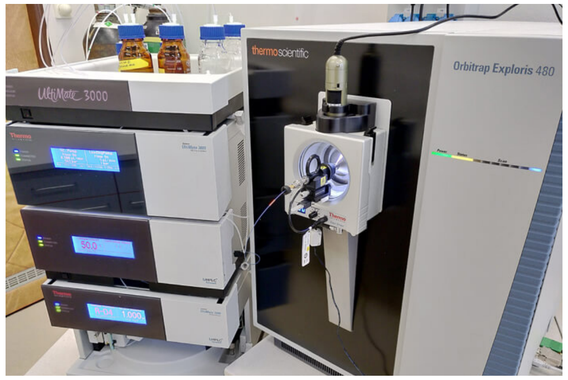
Miluše Hradilová

In the picture: 1. Hradilová Miluše | 2. Stehrer Thomas

Proteomics service laboratory jointly established by the IMG and IPHYS offers mass spectrometry-based proteomic analyses to the research groups of both institutes, and also to other academic and non-academic subjects. The paid-by-sample service includes the complete sample prep from various biological materials (e.g. cell lines, tissues, immunoprecipitation), LC-MS/MS of resulting peptides and the bioinformatic analysis. Please contact us to discuss your project including the experimental design and statistical considerations.

The laboratory is located in building E of IPHYS and is equipped with an Orbitrap Exploris 480 mass spectrometer coupled to nano UHPLC Ultimate 3000 liquid chromatograph.

Autosampler



UHPLC Ultimate 3000 liquid chromatograph coupled to Orbitrap Exploris 480 mass spectrometer

ADMINISTRATIVE SERVICES

ECONOMY DEPARTMENT

Kamila Dařinová

In the picture: 1. Knížková Klára | 2. Mičánková Bohumila | 3. Vokatá Pavlína | 4. Vašková Vlasta | 5. Linková Jiřina | 6. Nedvědová Irena | 7. Štorchová Emilie | 8. Dařinová Kamila | 9. Dařinová Monika | 10. Červenková Veronika | 11. Havlová Radka | 12. Hoferiková Lucie | 13. Veselý Lukáš | 14. Nezbedova Hana | 15. Málková Martina | 16. Peldová Erika | 17. Dlouhá Michaela | 18. Třísková Jitka | 19. Taský Roman

The Economy department manages the financial accounting and wage processing. We also oversee the Institute assets and participate in budgeting. We help new employees to find their way around IMG.

Our people will:

- process the received and issued invoices,
- handle the travel agenda and the calculation of travel allowances,
- prepare the documents required to correctly complete the tax return,
- prepare the overview of spending the grant funds.

Our other activities include:

- management of the tax matters related to IMG,
- set-up of control systems,
- preparation of documents for the Institute budget,
- defining sub-project and contract budgets, including their control methods,
- assistance to project managers with the preparation of financial reports.

The Economy Department also takes care of grant-related activities (grants agenda) and offers assistance with the preparation and submission of grant applications.

ADMINISTRATION TEAM

Martin Polák

In the picture: 1. Schmoranz Michal | 2. Macháčková Eva | 3. Chmelová Kateřina | 4. Škňouřilová Nikol | 5. Polák Martin | 6. Hladká Vladimíra | 7. Jonák Jiří

The Administration Team is responsible for all activities related to the processing of public tenders. We manage the records of IMG contracts and ensure their publication in the register of the Ministry of Interior of the Czech Republic. We are also responsible for the registration of IMG publications in the record keeping system for results of scientific work of the Czech Academy of Sciences (ASEP).

Our people will help you especially with:

- publication of orders and contracts,
- preparation of documentation for public tenders and their administration,
- coordination of technology transfer related activities.

BUILDING MAINTENANCE

Jana Boučková

In the picture: 1. Novotná Veronika | 2. Boučková Jana | 3. Jirout Miloš | 4. Dočekal Roman | 5. Švestka Michal | 6. Kulinkovský Jiří | 7. Řezník Roman

The Building Management is responsible for the operation of 7 buildings on the Krč campus: the main office and laboratory building, 3 animal facility buildings, the conference hall, the guesthouse, the kindergarten and the sports facility (total floor area 17,500 m²).

In these buildings, we provide – with the help of external companies – the operation and servicing of technologies, cleaning, repairs and modifications of the premises as well as their renovation. We also take care of the maintenance of the outdoor areas around IMG (area of 8,100 m²), including two ornamental ponds with seating.

Services of our people:

- for the international students and interns – arranging the accommodation in guesthouses on the Krč campus and handling the administration related to their stay in the Czech Republic,
- monitoring of freezing and refrigerating equipment in the buildings,
- record keeping and management of land and mobile phone lines,
- administration of the key system,
- technical support for scientific seminars and conferences.

We are also responsible for occupational health and safety (OHS) and fire protection (FP) trainings and car fleet maintenance.

OFFICE OF THE DIRECTOR

Petr Dráber

In the picture: 1. Takáčová Šárka | 2. Krausová Leona | 3. Dráber Petr | 4. Jarešová Jana | 5. Staněk David | 6. Chvojková Věra

Office of the Director, take care of the organisational and administrative agenda of the Institute Director, Deputy Director for research activities of the Institute and the Chairman of the IMG Council. The Office also prepares documents for the meetings of the Institute Management Board and is responsible for the administrative agenda of the Institute Secretary and the Secretary of the Supervisory Board.

People in the Office help with:

- administrative agenda related to the communication with the authorities of the Czech Academy of Sciences and grant agencies,
- translation of scientific and administrative texts into English,
- arranging legal services for your laboratory/facility.

In addition, Office provides registration and processing of all correspondence of the Institute and archiving of important documents. People in the Office also handle the agenda related to the management of the filing service and manage the IMG data mailbox.

Other activities of the Office include:

- preparation of annual reports and documents for the evaluation of the Institute activities,
- updating the internal website and preparing documents for meetings with the Institute units,
- organisation of staff training, researcher meetings, seminars, annual Institute conferences and many other internal and external activities.

INFORMATION TECHNOLOGIES

Petr Divina

In the picture: 1. Volf Tomáš | 2. Hurda Jan | 3. Škaroupka Petr | 4. Divina Petr | 5. Růžička Jiří | 6. Kohout Vojtěch | 7. Borovička Martin | 8. Šveňha Jan | 9. Žáček Michal | 10. Novotný Michal | 11. Mrázek Miroslav | 12. Kůs Michal | 13. Ježdík Ondřej | 14. Kopčan Juraj | 15. Rolník Michal

The IT department ensures the operation of LAN and WiFi networks at IMG, manages the institute servers and data storage infrastructure, and provides data backup and archiving.

To house the hardware, we run three modern data centres with air-conditioning control, uninterrupted power supply, environmental monitoring and automatic extinguishing system.

Our people will help you to:

- solve technical problems with computer equipment,
- select the appropriate hardware (especially computers and printers) for your work and connect it to the network,
- purchase licenses for scientific and office software,
- provide and operate the audiovisual equipment for trainings and conferences.

For the research and service groups, we also offer website development and programming of web applications to facilitate the day-to-day operation of the Institute. Moreover, we provide technical support for internal databases (e.g. the animal tracking system), manage the access control system to buildings and the CCTV system.

AWARDS AND HONOURS

2022

Radislav Sedláček	The František Běhounek Prize
Teije Corneel Middelkoop	The Lumina Quaeruntur Premium
Jiří Bártek	Honorary doctorate from Charles University for pioneering discoveries in the fields of cell biology and cancer research
Oksana Tsyklauri, Veronika Niederlová	Jaroslav Šterzl Award (Czech Immunological Society)

2021

Ondřej Štěpánek	EFIS Eastern Star Award for his project on the molecular basis of T-cell memory
Veronika Krchlíková	Josef Hlávka Award for the best students and graduates of Prague public universities, Technical University Brno, and young talented researchers of the Academy of Sciences of the Czech Republic
Kryštof Štafl	Best Retrovirology Paper by a Young Scientist Award 2021 (Retrovirology)
Veronika Horková	Jaroslav Šterzl Award (Czech Immunological Society)

2020

Václav Hořejší	The National Prize of the Czech Government "Česká hlava" (Czech Brains)
Matouš Vobořil	The Doctorandus Prize for natural sciences – Award of the VEOLIA company
Jiří Bártek	The Anders Jahre Medical Prize 2020 - Anders Jahre's medical award for outstanding research or results within medical sciences in the Nordic countries (University of Oslo)
Irena Jeníčková	ISTT Best Poster AwardBest Transgenic Technology Method Optimization (International Society for Transgenic Technologies)

BEST IMG PUBLICATIONS

Umístění

2022

1 Horkova V, Drobek A, Paprckova D, Niederlova V, Prasai A, Uleri V, Glatzova D, Kraller M, Cesnekova M, Janusova S, Salyova E, Tsyklauri O, Kadlecek TA, Krizova K, Platzer R, Schober K, Busch DH, Weiss A, Huppa JB, Stepanek O: Unique roles of co-receptor-bound LCK in helper and cytotoxic T cells. **Nat Immunol 2022**. [pubmed] [doi]

2 Zapletal D, Taborska E, Pasulka J, Malik R, Kubicek K, Zanova M, Much C, Sebesta M, Buccheri V, Horvat F, Jenickova I, Prochazkova M, Prochazka J, Pinkas M, Novacek J, Joseph DF, Sedlacek R, Bernecky C, O'Carroll D, Stefl R, Svoboda P: Structural and functional basis of mammalian microRNA biogenesis by Dicer. **Mol Cell 2022** *82(21): 4064-4079.e13.* [pubmed] [doi]

3 Cihlarova Z, Kubovciak J, Sobol M, Krejcikova K, Sachova J, Kolar M, Stanek D, Barinka C, Yoon G, Caldecott KW, Hanzlikova H: BRAT1 links Integrator and defective RNA processing with neurodegeneration. **Nat Commun 2022** *13(1): 5026.* [pubmed] [doi]

2021

1 Loubalova Z, Fulka H, Horvat F, Pasulka J, Malik R, Hirose M, Ogura A, Svoboda P: Formation of spermatogonia and fertile oocytes in golden hamsters requires piRNAs. **Nat Cell Biol 2021** 23(9): 992-1001. [pubmed] [doi]

2 Dupacova N, Antosova B, Paces J, Kozmik Z: Meis homeobox genes control progenitor competence in the retina. **Proc Natl Acad Sci U S A 2021** 118(12). [pubmed] [doi]

3 Klimešová K, Vojáčková J, Radivojević N, Vandermoere F, Bertrand E, Verheggen C, Staněk D: TSSC4 is a component of U5 snRNP that promotes tri-snRNP formation. **Nat Commun 2021** 12(1): 3646. [pubmed] [doi]

2020

1 Vobořil M, Brabec T, Dobeš J, Šplíchalová I, Březina J, Čepková A, Dobešová M, Aidarova A, Kubovčiak J, Tsyklauri O, Štěpánek O, Beneš V, Sedláček R, Klein L, Kolář M, Filipp D: Toll-like receptor signaling in thymic epithelium controls monocyte-derived dendritic cell recruitment and Treg generation. **Nat Commun 2020** 11(1): 2361. [pubmed] [doi]

2 Koslová A, Trefil P, Mucksová J, Reinišová M, Plachý J, Kalina J, Kučerová D, Geryk J, Krchlíková V, Lejčková B, Hejnar J: Precise CRISPR/Cas9 editing of the NHE1 gene renders chickens resistant to the J subgroup of avian leukosis virus. **Proc Natl Acad Sci U S A 2020** 117(4): 2108-2112. [pubmed] [doi]

3 [sdílené] Danek P, Kardosova M, Janeckova L, Karkoulia E, Vanickova K, Fabisik M, Lozano Asencio C, Benoukraf T, Tirado-Magallanes R, Zhou Q, Burocziova M, Rahmatova S, Pytlik R, Brdicka T, Tenen DG, Korinek V, Alberich Jorda M: β-catenin-TCF/LEF signaling promotes steady-state and emergency granulopoiesis via G-CSF receptor upregulation. **Blood 2020** 136(22): 2574-2587. [pubmed] [doi]

Hanzlikova H, Prokhorova E, Krejcikova K, Cihlarova Z, Kalasova I, Kubovciak J, Sachova J, Hailstone R, Brazina J, Ghosh S, Cirak S, Gleeson JG, Ahel I, Caldecott KW: Pathogenic ARH3 mutations result in ADP-ribose chromatin scars during DNA strand break repair. **Nat Commun 2020** 11(1): 3391. [pubmed] [doi]

CONFERENCES

2022

24. 11. 2022	EMBO Workshop on Research Integrity
18. 10. 2022	Join INFRAFRONTIER-IMPC Satellite Event at ICRI 2022
12. - 16. 10. 2022	32. Workshop on Retroviral Pathogenesis
10. 10. 2022	15th IMG PhD Conference
4. - 5. 10. 2022	Czech-BioImaging Annual Scientific Conference - Imaging Principles in Life 2022
28. – 30. 9. 2022	European Conference on Tetraspanins
15. – 16. 9. 2022	4th CCP Conference
28. - 31. 8. 2022	16th International Congress of Histochemistry and Cytochemistry 2022
27. 6. 2022	5th Czech Cilia Meeting
13. - 15. 6. 2022	ENBIK2022 – The National Bioinformatics Conference
2. - 3. 6. 2022	7th BAJ Symposium: "Gene Regulation, Stem Cells, and Leukemia"
26. – 27. 5. 2022	Annual IMG Conference

2021

3. 12. 2021	PATHBIO Satellite meeting: Reproducibility and Translatability in Research: the Path forward
26. – 27. 10. 2021	Czech-BioImaging Annual Scientific Conference - IMAGING PRINCIPLES OF LIFE 2021
1. 10. 2021	14th IMG PhD Conference
23. – 24. 9. 2021	IMPC 10th Anniversary Conference: Mouse Genetics and Genomic Medicine
16. – 17. 9. 2021	3rd CCP Phenogenomics Conference

2020

18. – 19. 11. 2020	Czech-BioImaging Annual Scientific Conference - IMAGING PRINCIPLES OF LIFE 2020
26. - 27. 10. 2020	11th European Zebrafish Meeting
2. 10. 2020	13th IMG PhD Conference
17. – 18. 9. 2020	2nd CCP Phenogenomics Conference




PhD PROGRAMME

The PhD programme at the Institute of Molecular Genetics (IMG) is part of a joint biomedical PhD programme organized by Charles University and several institutes of the Czech Academy of Sciences. Individual PhD programmes focus on specific topics and are organized by graduate boards established at Charles University. The PhD studies at the IMG focus on molecular, cell and developmental biology, immunology, genetics and virology. Currently, there are about 100 PhD students affiliated with the Institute.

The academic year at IMG starts with an **Academic Year Opening Meeting** in September, which is led by the IMG Director and is meant to introduce the new academic year and welcome the new PhD students. This is followed by the **IMG Bootcamp** in mid-October, which is dedicated to 1st year PhD students and their introduction to the student life at IMG. The IMG Bootcamp is focused on networking, motivation, and elementary skills students need during their PhD studies. The first half of November is dedicated to **Advances in Molecular Biology and Genetics**, a two-week seminar course, which most students have as one of their course requirements in their study plan. By the end of November, upon announcement of results of grant applications, preparation for selection of new PhD students starts. Once group leaders provide annotations of their open positions, a website is set up for **registration of prospective PhD candidates**. During January and February candidates can apply for the open PhD positions. Top ranking candidates will be selected for interviews, which will take place during the **PhD Interview Days** in March. In June, new PhD students will apply and register to Charles University and select their PhD boards.

PhD students are represented at IMG by two elected PhD candidates, which are active members of the IMG PhD committee. The **PhD representatives** are responsible for assessing the needs of the IMG PhD students and communicating with the IMG PhD committee and IMG management. PhD students run numerous activities independently and organize several scientific gatherings, including "**Hands on seminars**" and the **annual PhD conference**.

PEDAGOGICAL ACTIVITY

- = Field of study

PARTIAL COOPERATION AGREEMENTS WITH UNIVERSITIES

CHARLES UNIVERSITY

- Animal physiology
- Cell biology a pathology
- Immunology
- Molecular and Cell Biology, Genetics and Virology
- Developmental and Cell Biology
- Biochemistry and Pathobiochemistry

CHARLES UNIVERSITY

UNIVERSITY OF CHEMISTRY AND TECHNOLOGY PRAGUE

- Bioinformatics
- Synthesis and production of pharmaceuticals
- Biochemistry
- Molecular Biology

UNIVERSITY OF CHEMISTRY AND TECHNOLOGY PRAGUE

CTU

- Biomedical and clinical technology

CTU CZECH TECHNICAL UNIVERSITY IN PRAGUE

PH.D. SEMESTRAL COURSES

- Advances in Molecular Biology and Genetics
- Advances in immunology
- Epigenetics
- Grant application strategy course
- Innate Immunity
- Physiology of aging, cellular senescence and carcinogenesis
- Protein dynamics in development and cancer
- Supramolecular chemistry
- Systems Biology
- Transgenic models in physiology

BC./MSC. SEMESTRAL COURSES

- Advances in immunology
- Bioinformatics
- Case studies in bioinformatics
- Gene expression analysis
- Genomics: algorithms and analysis
- Grant application strategy course
- Chemical informatics
- Immunology
- Imunology
- Innate Immunity
- Medical Virology and Viral Pathogenesis
- Model organisms in developomental biology
- Molecular biology of cancer I
- Molecular mechanisms of cell cycle regulation
- Phylogenetics and Applied Genomics
- Physiology of aging, cellular senescence and carcinogenesis
- Protein dynamics in development and cancer
- Solving the three-dimensional structure of macromolecules
- Structural biology of the cell
- Structure and function of RNA
- Structure and function of the cytoskeleton
- Systems Biology

IMPRESSUM

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