Developmental and Cell Biology Board

The list of topics and supervisors who offer projects to applicants in the academic year 2020/2021

Consequences of radiation therapy in medulloblastomas

RNDr. Petr Bartůněk, CSc.

ID 225045

Medulloblastoma is the most common malignant brain tumor in children. In recent years, there has been a steady improvement in understanding the genetic and molecular heterogeneity of this tumor, which revealed at least four distinct subgroups of medulloblastomas. The project includes two major objectives devoted to elucidation of consequences of irradiation in particular molecular medulloblastoma subgroups. The research objective 1 is dedicated to characterization of functional impact of irradiation on medulloblastoma cells. We will explore potential differences in gene expression patterns, including expression levels of cell markers specific for cancer stem cells and differences in radioresistance in irradiated medulloblastoma cells compared with non-irradiated parental cells. In research objective 2, we will study how irradiation influences exosome-mediated signaling. We will ask if exosomes transmit prosurvival effects by promoting proliferation, migration and radioresistance of medulloblastoma cells and thus increase resistance against radiation therapies in medulloblastoma. The aims of the project
(1) the functional impact of therapeutic radiation on medulloblastoma cells
(2) how irradiation influences exosome-mediated signalling.

Cytoskeletal regulation through phase transitions of intrinsically-disordered proteins

Marcus Braun, Ph.D.

ID 212062

Tau is an intrinsically-disordered microtubule-associated protein, predominantly localized in neurons, which is involved in neurodegenerative disorders, such as the Alzheimer's disease. Tau is known to stabilize microtubules and regulate the function of many other microtubule-associated proteins. What underlies these regulatory mechanisms on the molecular level is not fully understood. It has been shown recently that tau can form compartments through liquid-liquid phase separation in solution and ordered domains on the microtubule surface presumably also through phase separation. How do these processes regulate other cytoskeletal proteins and processes is however unknown.

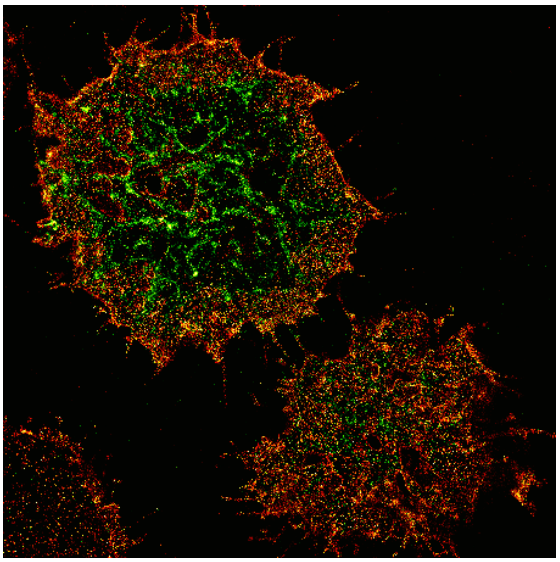
In this project we will explore the regulatory roles of the ordered domains of tau and other intrinsically disordered cytoskeletal proteins emerging on the surface of cytoskeletal filaments. We will generate these domains on filaments in vitro and probe i) the interactions between domains of different intrinsically disordered proteins, and ii) the interactions of other proteins associated with cytoskeletal filaments, such as motor proteins or filament-severing enzymes, with these domains. Additionally / alternatively, we will explore the roles of these domains in the stabilization of cytoskeletal filaments and in generation of forces between cytoskeletal filaments. We will image the explored systems with single molecule resolution using total internal reflection fluorescence (TIRF) microscopy and probe the generated forces using optical tweezers. With this project we aim to clarify some of the regulatory mechanisms mediated by tau and other unstructured cytoskeletal proteins.

Unravelling molecular surface nanotopography of immune cells by advanced microscopy

Mgr. Marek Cebecauer, Ph.D.

ID 212224

Lymphocytes co-ordinate immune responses against infections and cell transformations. Main immune receptors (e.g., TCR, BCR) trigger the essential signals to activate these cells but a handful of co-stimulatory receptors (e.g., CD2, CD20, integrins and PD-1) regulate the output of these processes. The mechanisms co-ordinating the action of these receptors remain incompletely understood. The information about the spatio-temporal organisation of the receptors and associated signalling events is largely missing. In our laboratory, we have recently discovered that CD4 co-receptor accumulates at the tips of T cell microvilli whereas its regulatory phosphatase, CD45, segregates to the remaining parts of the plasma membrane. This discovery indicates the importance of nanotopography in the organisation of receptors on T cells before and during recognition of antigens. Based on these results, we are currently studying previously over-looked, TCR-independent role of CD4 in T cell activation and homeostasis. However, the activity of immune receptors can be modulated by other surface receptors, nanoscopic organisation of which is unknown. We plan to map distribution (3D, nanoscopic) of CD2, CD20, integrins and PD-1 on the surface of lymphocytes, especially with respect to the pathogenic processes. The project will include the use of the state-of-the-art super-resolution microscopy (co-developed in our laboratory), functional analysis of living lymphocytes and the recombinant DNA technologies.



Exploring mechanisms of DNA replication restart upon collisions between transcription and replication

RNDr. Jana Dobrovolná, Ph.D.

ID 223125

Transcription-replication collisions (TRCs) represent a significant source of genomic instability in cells experiencing DNA replication stress. Although there is a great deal of knowledge about the strategies that cells evolved to avoid TRCs, understanding of how a replication fork restarts DNA synthesis upon a TRC remains elusive. Our recent studies have shown that replication restart upon R-loop-mediated TRCs relies on MUS81 endonuclease, RAD52 single-strand annealing protein and DNA ligase 4 (LIG4). In this project, we aim to use a proteomic approach to identify and functionally characterize new factors involved in this process. The student will also undergo short-term trainings at the Institute of Molecular Cancer Research of the University of Zurich where he/she will be exposed to front-line research in the field of DNA repair and cancer.

Single-cell analysis in systems immunology – an application of novel unsupervised tools in infectious diseases and cancer

RNDr. Karel Drbal, Ph.D.

ID 212586

The objective of this project is an application of novel analytical tools for the large transcriptomic and proteomic datasets in clinics. This is in principle unsupervised topological data analysis (TDA) based on clustering, which allows for immediate statistical data evaluation and visualization using dimensionality reduction down to 2D. We are going to optimize parameters, achieve minimal data distortion and maximal reproducibility as well. Galaxy platform is a central cloud environment, however, a deep knowledge of R/Python is essential (C++ programming is a bonus). In turn, this brings a completely new understanding of existing scientific data in general and allows for the reinterpretation and discovery of new relationships.

We will focus on clinical datasets in patients suffering from infectious or tumor diseases. An inherent part of the workflow is data collection and database maintenance. A prediction of directional causal relationships will be finally validated in available zebrafish and/or medaka models in our laboratory at the level of transcriptome and proteome.

Recently, an excessive boost in the development of analytical bioinformatic algorithms allows biologists to mine the available datasets originating from single cells in a completely unsupervised manner for the first time. The human body is composed of around 30 trillion cells and the objective of this application is the use of novel dimensionality reduction, trajectory inference, and clustering algorithms in order to decode their directional relationship in the field of systems immunology.

Our research focuses on dynamic systems of immune response monitoring in patients suffering from infectious diseases – tuberculosis or borreliosis – as well as various solid tumors – mainly bladder cancer. Under these pathological conditions, activation of immune cell subsets of both innate and adaptive systems regulate the outcome of the disease. A single-cell oriented statistical data evaluation and visualization finally stratify each patient. We are going to optimize the parameters of the non-linear computational methods in order to preserve data distribution and maximize reproducibility.

An inherent part of the workflow is the collection of genomic/transcriptomic/proteomic data and patient database maintenance. The predictions of directional causal relationships will be validated in available zebrafish and/or medaka models after multiparametric flow cytometry and cell sorting in our laboratory at the level of gene and cellular networks. A collaboration with local clinical partners (TH, HNB, Prague) and computation centers (IDA FEE CTU [1], IOCB CAS [2], Prague) are backed by recent publications. International collaboration within LifeTime consortia will be an inherent part of the project.

As stated above, deep knowledge of R and Python (C++ is a bonus) and immunology is an essential profile of a successful candidate. Optionally, the experience with cytometry and/or microscopy and the knowledge of one of the experimental models is a plus. Two major goals of this Ph.D. position are 1/ the integration of existing tools into a Galaxy pipeline or development of a standalone application and 2/ the identification of cellular biomarkers of either latent TB infection, late-stage Lyme disease or bladder cancer stem cells.

1. Dvorakova, E. et al. Bioinforma. Res. Appl. ICBRA 2019 (2019). at http://ida.felk.cvut.cz/zelezny/pubs/icbra2019.pdf

2. Kratochvíl, M. et al. bioRxiv 496869 (2019).doi:10.1101/496869

Characterization of the knock-in mini-pig model of Huntington's disease and identification of pre-manifest biomarkers

Ing. Zdenka Ellederová Ph.D.

ID 223364

The aim of this thesis will be to characterize the 85Q knock in Huntington's disease minipig model and identify pre-manifest biomarkers. Mutated huntingtin, neurofilament light protein, and a panel of cytokines, different markers of metabolism, mitochondria biogenesis and oxidative stress will be measured in blood, CSF and cells isloated from alive animals. Furthermore, some animals will be sacrificed at different ages and biochemical and histological analysis of tissues will be performed. Markers of neurodegeneration such as medium size spiny neuron marker (DARPP32), marker for activated microglia (IBA1), an astrocyte marker (GFAP), marker associated with cell apoptosis (Cas3), and also changes in cellularity and myelinization will be analyzed together with mutated huntingtin accumulation and detection of its forms.

Above mentioned experiments will contribute to characterization of this unique animal model of HD, and finding important biomarkers for potential preclinical testing.

Potential therapeutic approaches to the treatment of Huntington's disease

Ing. Zdenka Ellederová Ph.D.

ID 223365

Huntington's disease (HD) is an autosomal dominant neurodegenerative disease which is caused by the expansion of the CAG repeat in the translated sequence of the huntingtin (HTT) gene. Mutated HTT causes atrophy of medium spinal neurons. The brain pathology appears already before the clinical onset of the disease. There are currently no disease-modifying therapies available to treat HD patients.

The aim of this thesis will be to test a preclinical approach to treat HD. At first terapeutic vectors: siRNA for silencing mHTT and siRNA for depleting the RNA binding protein PTB for an efficient single-step conversion of astrocytes into functional neurons will be generated and tested in vitro. Then ethylene imine (PEI) biopolymer coated magnetic nanoparticles (MNPs) delivery system coupled with therapeutic vectors will be tested . The delivery efficacy of PEI-MNPs-siRNA complexes and the efficacy of reprogramming of astrocytes into neurons and the down regulation of mHTT protein will be tested in vitro by immunocytochemistry, Western blot and QPCR. Subsequently, we will test this approach in vivo using HD mice to provide a solid background for testing this approach in HD pigs, a large animal model generated by us.

The project will contribute to the development of safe gene therapy approaches for HD.

The roles of RNA structures in the regulation of splicing

doc. RNDr. Petr Folk, CSc.

ID 222547

Recent whole genome studies showed that all RNAs including pre-mRNAs are extensively structured and that the structures change with cultivation conditions or cell types. Molecules of pre-mRNA, and introns in particular, can be viewed as polymorphic structures rather than passive carriers of sequence information (1,2). As such, they can regulate the accessibility of splice sites or affect the maturation pathway of spliceosomes. RNA folding in vivo is influenced by concentrations of ions, temperature, binding of proteins, crowding effect of the microenvironment, activities of helicases, and post-transcriptional base modifications (3,4). All the factors act in concert and change with time, turning pre-mRNPs into a changing assembly of secondary, super secondary and tertiary structures. Examples of tertiary RNA structures have been already documented in detail (5,6).

Previously, we described a splicing-based regulatory relationship between ribosomal genes RPL22A and RPL22B of Saccharomyces cerevisiae. We demonstrated that the regulation required Rpl22 protein binding to a highly structured intronic region of pre-mRNA (7). This project addresses the question of how can pre-mRNA structures, in response to signals such as ribosomal protein binding, hinder or aid spliceosome assembly and/or direct the pre-mRNA toward degradation. We want to test the hypothesis that structural features of introns provide the interface between the signals in cis and the spliceosome. The student will use techniques of yeast microbiology and molecular genetics, high throughput selection procedures to isolate mutations affecting RNA function, as well as in vitro techniques to study RNA-protein binding.

Regulated splicing can modulate gene expression in yeast during, e.g., meiosis or nutrient limitations (8,9). Our results can thus find practical application, because they can provide novel tools for manipulations in biotechnology and synthetic biology.

1. P. C. Bevilacqua et al., Annu. Rev. Genet. 50, 235–66 (2016).

2. M. B. Warf, J. A. Berglund, Trends Biochem. Sci. 35, 169–78 (2010).

3. S. Rouskin et al., Nature. 505, 701–5 (2014).

4. K. Kaushik et al., BMC Genomics. 19, 147 (2018).

5. D. K. Hendrix et al., Q. Rev. Biophys. 38, 221–43 (2005).

6. D. Antunes et al., Front. Genet. 8, 231 (2018).

7. K. Abrhámová et al., PloS One. 13, e0190685 (2018).

8. E. M. Munding et al., Mol. Cell. 51, 338–48 (2013).

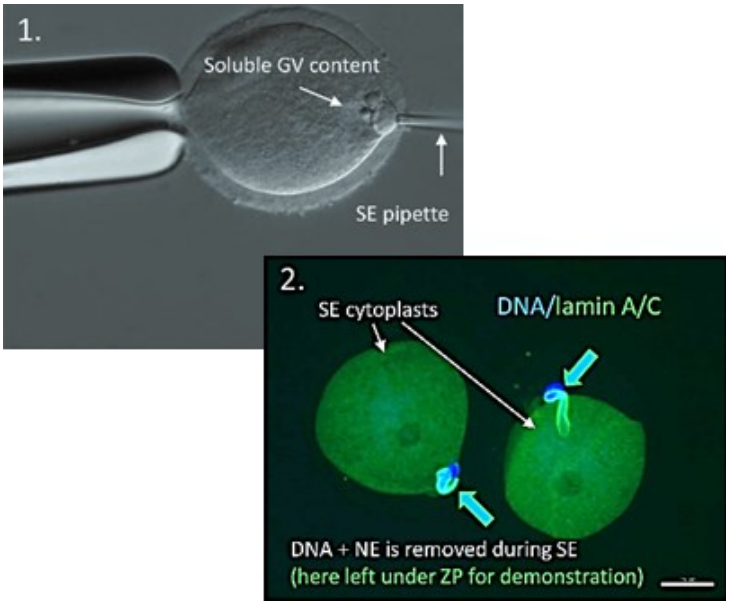
9. J. T. Morgan et al., Nature. 565, 606–11 (2019).

Understanding the factors critical for successful paternal genome remodelling

Mgr. Helena Fulková, Ph.D.

ID 223126

The nucleus is a hallmark of nearly all eukaryotic cells and central to their function. Aberrations in the nuclear composition and structure are linked to diseases such as cancer. The project is focused on how cells build a nucleus from different genomic substrates and what is the consequence of nuclear dysregulation, with the ultimate goal of elucidating the function of the nucleus as an essential cellular structure. While many theories exist, it is extremely difficult to assess these in tissue culture cells. Early embryos offer a unique opportunity to study these phenomena: Oocytes build a nucleus from a practically naked DNA (sperm), and also two morphologically and functionally different nuclei (maternal and paternal) co-exist in a common cytoplasm after fertilisation. Nevertheless, both parental genomes must be extensively (epigenetically) remodelled. The egg components necessary for the pronucleus construction and genome remodelling remain to be characterized in detail. With the help of micromanipulations we plan to analyse the essential egg components necessary for the formation of fully functional pronuclei. In contrast to egg extracts, this system offers an unprecedented chance to rigorously test the consequence of targeted nuclear dysregulation through the ability of embryos to give rise to live animals.



Broad spectrum antibiotic discovery

Mgr. Klára Hlouchová, Ph.D.

ID 223298

There remains a need for novel antibiotics, particularly those directed against multi-resistant Gram-negative bacteria. Common species of the genera Acinetobacter, Pseudomonas and many Enterobacteriaceae are currently resistant to all known antibiotics. Unless antibacterial development is re-energized, there is a risk that a growing proportion of hospital infections become effectively untreatable.

A high throughput search is proposed for the discovery of novel broad-spectrum antibiotics. The screen will use large libraries of synthetic peptides to target bacterial ribosomal assembly and function. The binding sites of most widely used antibiotics are concentrated at and around functional hot spots of the small 30S and the large 50S subunit of the bacterial ribosome. These sites are often dominated by elements of the rRNA but still require protein activity. The libraries will be formed from mutated variants of natural ribosomal proteins necessary for the formation and activity of both the small and the large ribosomal subunit. The mutation strategy involves an increased likelihood of binding to the ribosome target, loss of function mutations.

The outcome of the project is the expected discovery of novel antibiotics leads, which target by design, gram negative, resistant and otherwise difficult to treat microbes.

The candidate should have at least basic wet-lab skills (ideally previous experience with bacteria cultivation and protein interaction characterization). Basic bioinformatic and large data analysis skills are of advantage.

From random sequences to vital proteins

Mgr. Klára Hlouchová, Ph.D.

ID 223306

It is widely assumed that new proteins derive from old proteins, via duplication and adaptation of one of the copies or by combining smaller, already viable fragments with a well-defined structure. However, it recently became clear that (i) many functional proteins do not assume well-defined structures, (ii) proteins from random sequences can adapt and assume novel functions too, and (iii) in modern organisms many functional proteins emerge ''de-novo'', i.e. from previously non-coding DNA regions lacking any prior selection. This project will thus explore the boundaries between viable and non-viable protein sequences in the context of contemporary forms of life.

To mimic the process of “de-novo” protein evolution, random protein sequences will be used as proxies for spontaneously emerging polypeptides. Initially, large random sequence libraries (>1012 variants) will be generated and expressed using modern methods of synthetic biology (automated design, DNA assembly, expression by cell-free translation systems) and using a semi-automated pipeline, the library expression/solubility profile will be evaluated in a 96-well format. Expression/solubility characteristics, together with corresponding features set will serve as an input for machine learning optimization. Sequential optimization using prediction/analysis cycles will provide a trajectory of features (such as amino acid composition and secondary structure properties) important for proteins to become tolerated and viable in contemporary life. Such “manual” will help to define what sets apart viable protein sequences from artificially generated random sequences and will find applications in protein design initiatives.

The project will then aim at studying functional potential of optimized random sequences, using high-throughput selections for protein interaction and catalysis through international collaboration of the group.

The candidate for this project should ideally have both wet-lab and bioinformatic basic skills.

Search for viability principles in protein sequence space – an in vivo approach

ID 223584

Mgr. Klára Hlouchová, Ph.D.

The aim of this thesis will be exploration of random sequence viability as a parallel to de novo gene birth in eukaryotic systems. It will rely on introduction of random protein libraries into Sacharomyces cerevisiae, as an eukaryotic model organism. The methodology of design and synthesis of random sequence libraries has been recently acquired in the project supervisor group (manuscript describing library design algorithm and proof-of-concept has been submitted). Genes encoding 90 amino acid long polypeptides will be subcloned in configurations (such as tags and fusions) specific for the following research goals: (i) evaluation of expression/solubility, (ii) biological fitness and half-lives, and (iii) phase separation behaviour for 500 selected transformants. The results of the characterization will be compared to behavior or selected de novo genes from Sacharomyces cerevisiae from a collaborating research group in Germany.

The findings of this thesis should therefore test whether de novo proteins are more evolvable than random sequences and seek differences in their sequence parameters, contributing to understanding how nature crosses the boundary of protein sequence viability in context of today's life.

The role of phosphoinositides in spatiotemporal regulation of nuclear processes

prof. RNDr. Pavel Hozák, DrSc.

ID 221781

Phosphoinositides (PIPs) are recognized as regulators of many nuclear processes including chromatin remodeling, splicing, transcription, and DNA repair. These processes are spatially organized in different nuclear compartments. Various nuclear compartments are formed by entropy-driven mechanism - phase separation. The surface of such membrane-less structures spatiotemporally coordinates complex nuclear processes. The integration of PIPs into the surface of nuclear structures might therefore provide an additional step in their functional diversification by controlling the localization of different components, in a similar way as PIPs do in membranous cytoplasmic environment. This project focuses on deciphering the molecular mechanisms of various PIPs in establishing a dynamic nuclear architecture. In this project PhD candidate will characterize the PIPs-containing nuclear structures by combination of lipidomics, proteomics (quantitative MS), molecular biology (e.g. CRISPR/Cas9), biochemical and advanced microscopy (e.g. confocal, SIM, STED, FRAP) methods. The project is supported by funding from the Grant Agency of the Czech Republic.

Chemical biology tool for the analysis of proteases of human pathogens: from microbes to Alzheimer

doc. RNDr. Jan Konvalinka, CSc.

ID 225100

Doktorandka bude vyvíjet nové chemicko biologické nástroje pro kvantifikaci, visualisaci a cílení enzymů důležitých pro patogenesi významných lidských chorob a pro hledání jejich účinných inhibitorů. Ve své práci bude využívat metody, nově vyvinuté ve skupině Dr. Jana Konvalinky na UOCHB AV ČR. Jednou z nich je metoda DIANA pro kvantifikaci proteinů využívající konjugátů inhibitorů těchto enzymů s oligonukleotidy. Vazba je pak detekována a velmi přesně kvantifikována pomocí qPCR. Další metodou jsou synthetickéá mimetika protilátek zvaná iBody, polymerní konjugáty založené na hydroxypropylmethakrylamidovém kopolymeru (HPMA), dekorovaném nízkomolekulárními ligandy příslušného enzymu, které umožňují jeho visualisaci, isolaci a/nebo zacílení.

Modelem, na kterém bude tyto nástroje používat, budou cysteinové proteasy z Porphyromonas gingivalis. Tato bakterie, způsobující záněty dásní pacientů s pardentosou, byla identifikována v mozcích pacientů s Alzheimerovou chorobou a velmi nedávno byla postulována hypothesa o možné etiologické souvislosti patologie Alzheimerovy choroby se zánětem způsobeným P. gingivalis. Cysteinové proteasy P. gingivalis jsou důležitým faktorem pathogenity viru a jejich kvantifikace a identifikace může být důležitým nástrojem pro studium a experimentální terapii Alzheimerovy choroby.

Doktorandka bude v rámci své disertační práce připravovat rekombinantní proteasy z P. gingivalis, purifikovat je a charakterisovat. Navrhne a ověří metody na testování jejich aktivity nebo správného složení (foldingu). Ve spolupráci s medicinálními chemiky na UOCHB navrhne, otestuje a validuje metodu na kvantifikaci těchto enzymů jak in vitro, tak na buněčné úrovni. Dále bude identifikovat jejich nízkomolekulární ligandy, a to mj. i testováním rozsáhlé knihovny sloučenin na UOCHB AV ČR. Bude testovat aktivitu polymerních konjugátů dekorovaných těmito ligandy pomocí biochemických metod (SPR, ELISA) a charakterisovat jejich interakci s cílovými enzymy jak pomocí rekombinantních proteinů, tak i tkáňových řezů experimentálních myších modelů.

Práce bude kryta běžícími granty ve skupině J. Konvalinky na UOCHB AV ČR, zejména finanční podporou Gilead Science and IOCB Research Center Prague (do r. 2023) a grantem Evropské komise ERA-NET JPND na studium souvislosti pathogenese Alzheimoreovy choroby a paradentosy ("Alzheimer's disease as a co-morbidity of chronic periodontitis with Porphyromonas gingivalis as a causative link between both diseases; Gums and Brains") ve spolupráci s laboratořemi v Německu, Dánsku, Polsku a Norsku (2020-2023).

Literatura:

Dominy S.S. et al. (2019) Porphyromonas gingivalis in Alzheimer's disease brains: Evidence for disease causation and treatment with small-molecule inhibitors. Sci Adv. 5 (1): eaau3333,

DOI: 10.1126/sciadv.aau3333.

Ashraf G.M. et al. (2018) The Possibility of an Infectious Etiology of Alzheimer Disease. Mol Neurobiol. doi: 10.1007/s12035- 018-1388-y.

Šácha, P. et al. (2016). iBodies: Modular Synthetic Antibody Mimetics Based on Hydrophilic Polymers Decorated with Functional Moieties. Angew. Chem. Int. Ed. Engl. 55, 2356-2360

Navrátil, V. et al. (2017) DNA-linked Inhibitor Antibody Assay (DIANA) for sensitive and selective enzyme detection and inhibitor screening. Nucl. Acids Res. 45 (2), e10 DOI: 10.1093/nar/gkw853

Characterization molecular mechanisms causing intestinal cancer at single-cell level

RNDr. Vladimír Kořínek, CSc.

ID 222955

Intestinal carcinoma is one of the most frequent types of cancer in Western countries. Intestinal carcinomas evolve throughout a series of mutational events that produce considerable tumor cell heterogeneity. It is presumed that tumors in the initiation stages of the tumorigenic process - so-called microadenomas - are composed of highly proliferative cells displaying similar molecular and cell biology features. However, our recent results indicate that even early neoplastic lesions formed in the colon of experimental mice are composed of highly heterogeneous cell populations reminding of cell types present in the healthy colon epithelium. We aim to reveal the molecular mechanisms related to the observed tumor cell phenotypic heterogeneity. The research includes work with mouse transgenic models, analysis of gene expression at the single-cell level, and evaluation of the tumor cell epigenetic status. A part of the study will be performed using intestinal organoids derived from healthy or tumor tissue.

Our laboratory is looking for a new, highly motivated team member with interest in biomedical research. The candidates should hold a Master degree in cellular and molecular biology, genetics or related fields. We expect that the prospective candidate is willing to learn advanced research techniques that include work with mouse tissues and human patient samples. We offer a friendly environment well-adjusted to the proposed research topic and decent financial conditions.

Evolution of eyes: insight from photoreceptors of invertebrate chordate amphioxus

RNDr. Zbyněk Kozmík, CSc.

ID 223147

Project aims to provide new insight into evolution of animal eyes using invertebrate chordate amphioxus as a laboratory model. Project will specifically focus on amphioxus photoreceptors that are not associated with pigment cells: Joseph cells, bearing similarity to intrinsically photosensitive retinal ganglion cells of vertebrates, and lamellar body that is likely homologous to vertebrate pineal organ. The methods used will include basic bioinformatics analysis, gene isolation, gene expression studies by transcriptomics, whole-mount in situ hybridization and immunohistochemistry, biochemical characterization of photosensitive proteins (opsins) in vitro, characterization of selected gene knockouts produced in the lab by CRISPR/Cas9 system, and behavior visual tests (in collaboration with laboratory in Vienna).

Cellular interactions in tumour stroma and their effects on tumour expansion

Mgr. Helena Kupcová Skalníková, Ph.D.

ID 223199

Tumours are formed not only by malignant cells, but also stromal cells (fibroblasts, vascular endothelial cells and others), immune cells and extracellular matrix. Interplay among such members of tumour microenvironment predicts the tumour growth as well as response to therapy. Current therapies are mostly targeted against the malignant cells. Nonetheless, therapeutic targeting of side components of the tumour microenvironment may have far-reaching effects also on the intrinsic malignant cells.

The cells in the tumour stroma communicate by direct contact, variety of autocrine and paracrine molecules or extracellular vesicles released to tissue as well as blood and lymph. The aim of this project is to characterize secreted factors, including extracellular vesicles, in microenvironment of melanoma and their effects on tumour growth and prognosis using human cells and porcine melanoma model MeLiM.

Methods: cell culture, blood and tissue collection from animals, isolation and characterization of extracellular vesicles, flow cytometry, biochemical techniques, immunological methods (Luminex, ELISA), proteomics (mass spectrometry, bioinformatics).

Regeneration potential of Sertoli cells

doc. RNDr. Ing. Vladimír Krylov, Ph.D.

ID 223188

Regeneration is important field of modern medicine, because of a limited regeneration capacity of adult mammals, in contrast to amphibians. A key factor behind regeneration capacity is different immune response to injury. Stem cells has been proposed to replace regeneration deficit in mammals. Unfortunately, clinical outcomes of stem cell transplantation in regenerative medicine remain poor, so there is a continuous demand for cells with better capacity to restore damaged tissues function. In recent years, immune-tolerable stem-like Sertoli cells (SCs) have been introduced as a potential solution. PhD applicant would study a behavior and gene expression of isolated and defined SCs after transplantation to allo- and xenogeneic recipients with or without injury using two or three evolutionary distant species, Xenopus and Mus musculus or rat, which represent full and partial regenerative systems. The injury would include the cuting the part of tadpole´s tale , surgery of adult frog heart removing the part of its apex or infarction induction in mouse or rat models. Regeneration properties of SCs will be evaluated and changes of gene expression among host and recipient cells will be studied at a single cell level to elucidate molecular mechanism underlying their differentiation and regeneration and immune response.

Molecular pathophysiology of myeloproliferative blood neoplasms

Ing. Lucie Láníková, Ph.D.

ID 223244

The aim of this project is the identification of the new genetic predispositions to myeloproliferative neoplasms (MPN) and their in vitro/vivo characterization, especially congenital weakly activating mutations in the JAK/STAT pathway. We propose to create a mouse model of prevalent germ-line Jak2 mutation and its cross-breeding with relevant MPN-like models. Reliable characterization of predisposing genetic variants based on analysis of the mouse model is the key step for improving the clinical diagnosis and prevention of hematologic malignancies. Recently we published a concept of protection mechanisms that guard myeloproliferative progenitors from cell-intrinsic and cell-extrinsic DNA damage and thus DNA-damage response (DDR), facilitating creation of a barrier preventing cell cycle arrest, myelofibrosis and rapid malignant transformation. We plan to test whether and how this process can be targeted in preventing full leukemia transformation from different preleukemia disease states.
Informace ke studiu/Suggested reading:
Stetka J. et al. Addiction to DUSP1 protects JAK2V617F-driven polycythemia vera progenitors against inflammatory stress and DNA damage, allowing chronic proliferation. Oncogene. 2019;38(28):5627-5642.
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Cytoskeletal mechanics of axonal pathfinding

RNDr. Zdeněk Lánský, Ph.D.

ID 222733

Lab profile: Cytoskeletal networks form the internal dynamic scaffold of living cells essential for key cellular processes, such as cell division, cell motility or morphogenesis. Our aim is to understand how the individual structural elements of the cytoskeleton mechanically cooperate to drive these cellular processes.

We use reconstituted cytoskeletal systems to study the system's self-assembly and dynamics. Central to our approach are imaging, manipulation and force measurement techniques with single molecule resolution.

Project description: During the ontogenetic formation of the nervous system, growth cones of young axons navigate through a maze of physical boundaries to establish synapses. Growth cone progression is propelled by the dynamics of the actin cytoskeleton, while the direction of growth is governed by the dynamics of microtubules. Intricate crosstalk between microtubules and actin filaments thus must take place for fully functional growth cone steering. The aim of the project is to explain how this crosstalk is mediated and how it regulates axonal pathfinding.

Candidate profile: We are looking for an enthusiastic PhD student motivated to work on cross-disciplinary projects. The candidate should hold a master's degree in (bio)chemistry, (bio)physics, molecular/cellular biology or an equivalent field.

Regulatory roles of the microtubule envelope

RNDr. Zdeněk Lánský, Ph.D.

ID 222732

Lab profile: Cytoskeletal networks form the internal dynamic scaffold of living cells essential for key cellular processes, such as cell division, cell motility or morphogenesis. Our aim is to understand how the individual structural elements of the cytoskeleton mechanically cooperate to drive these cellular processes.

We use reconstituted cytoskeletal systems to study the system's self-assembly and dynamics. Central to our approach are imaging, manipulation and force measurement techniques with single molecule resolution.

Project description: Modulating the accessibility of the cytoskeletal filaments for the filament-associated proteins is one of the fundamental regulatory mechanisms in the cytoskeleton. Unstructured microtubule-associated proteins, such as the Alzheimer's disease-associated protein tau, can form cohesive envelopes around microtubules, selectively modulating the microtubule accessibility by locally excluding specific proteins from the microtubule surface while recruiting others. The aim of the project is to explain the envelope formation and its regulatory and (patho)physiological roles.

Candidate profile: We are looking for an enthusiastic PhD student motivated to work on cross-disciplinary projects. The candidate should hold a master's degree in (bio)chemistry, (bio)physics, molecular/cellular biology or an equivalent field.

Cytoskeletal crosslinkers in the growth cone of neuronal cells

RNDr. Lenka Libusová, Ph.D.

ID 223308

The newborn neuronal cells develop and interconnect their processes in order to establish a functional neuronal system. The progression of individual axons is propelled by the cooperation between microtubules and actin network at the axonal growth cone. However, the role of proteins mediating this crosstalk remains poorly defined.

This project aims to visualize, analyze and explain the mechanism of the growth cone steering. Several candidates among actin-microtubule crosslinkers will be studied by advanced live-cell microscopy on neuronsdifferentiated from the transgenic iPSC (induced pluripotent stem cells)as well as by in vitro reconstitution approaches. The function, dynamics and time-scale of their interaction with cytoskeletal networks will be evaluated.

Dinucleoside polyphosphate RNA caps in innate immune cells

Ing. Hana Macíčková Cahová, Ph.D.

ID 223131

Recent discovery of RNA caps such as NAD and CoA lead to the reassessment of RNA structure in all types of cells. In our search for new RNA modifications, we have discovered a brand-new class of 5‘caps - dinucleoside polyphosphates (NpnN) in bacteria. NpnNs are ubiquitous second messengers from bacteria to eukaryotes. Especially, Ap4A plays a crucial role in the signalling of mast cells and its intracellular concentration significantly increases upon IgE activation. The task of the thesis is to reveal whether dinucleoside polyphosphates are a part of RNA in innate immune cells and what role they play in RNA during the immune response.

Molecular mechanisms of pathogenicity in ATP synthase disorders

RNDr. Tomáš Mráček, Ph.D.

ID 211570

Mutations in mitochondrial FoF1 ATP synthase lead to severe inborn errors of metabolism. As is the case with other mitochondrial diseases, one of the striking features is the tissue specificity of symptoms associated with mutations in individual subunits. Thus, mutations in TMEM70 or ATP5E present primarily as myopathies, while Usmg5 patients present with neurological disorders. While the primary biochemical features are generally characterised, mechanisms dictating tissue specificity are still poorly understood.

Recently, we have developed animal models for defects in TMEM70 as well as Usmg5. The aim of this project is to explore differences in tissue presentation as well as compensatory or regulatory mechanisms involved to mitigate pathogenic phenotype. The project should aim beyond the biochemical characterisation of mitochondrial function and dig further into the adaptations occurring at the whole body level to understand the role of ATP synthase in modulation of metabolic plasticity.

This project should take the advantage of wide array of phenotypisation techniques available at the Institute of Physiology and adapt them for the use on mitochondrial models.

Candidate's profile (requirements):MSc or MD degree in (animal) physiology or similar. Candidates should have a good record of accomplishment in physiology and biochemistry. Willingness to work with laboratory animals is requirement, previous experience strong asset.

Relevant publications:

1. Kovalčíková J, Vrbacký M, Pecina P, Tauchmannová K, Nůsková H, Kaplanová V, Brázdová A, Alán L, Eliáš J, Čunátová K, Kořínek V, Sedlacek R, Mráček T, Houštěk J.: TMEM70 facilitates biogenesis of mammalian ATP synthase by promoting subunit c incorporation into the rotor structure of the enzyme. FASEB J. 2019 Dec;33(12):14103-14117

2. Vrbacky M, Kovalcikova J, Chawengsaksophak K, Beck IM, Mracek T, Nuskova H, Sedmera D, Papousek F, Kolar F, Sobol M, Hozak P, Sedlacek R, Houstek J. Knockout of Tmem70 alters biogenesis of ATP synthase and leads to embryonal lethality in mice. Hum Mol Genet. 2016;25(21):4674-85

Mitochondrial and nuclear DNA cross-talk and its impact on metabolic phenotype and innate immunity

RNDr. Alena Pecinová, Ph.D.

ID 223242

Recently, metabolic syndrome has been associated with chronic, low-grade systemic inflammation, increased immunogenetic susceptibility and rise in circulating immune markers. Interestingly, incidence of some of the hallmarks of metabolic syndrome (e.g. type 2 diabetes) differs among ethnicities - while part of the variability may be explained by different quality of care between ethnic groups, others seem to stem from genetic diversity. An important role may be played by physiological genetic diversity of maternally inherited mitochondrial DNA. Remarkably, mitochondria have also been demonstrated to trigger host immune response, namely by activating innate immune system.

In the current project, we will utilize the model of rat conplastic strains with several mtDNA haplogroups present on identical nuclear background. We will test the hypothesis that the naturally occurring mtDNA diversity influences systemic inflammation status and further explore pathways involved in this process. Subsequently, the propensity towards development of metabolic syndrome and innate immunity response during metabolic challenge will be tested.

Plasticity of cancer cells invasiveness and its targeting by migrastatic drugs

doc. RNDr. Daniel Rösel, Ph.D.

ID 212240

Only for students enrolled in STARS program! The malignancy of solid cancer is mainly caused by the ability of tumor cells to form metastases. The crucial step during metastasis is the invasion of the cancer cells through the ECM. To achieve this, cancer cells can utilize the protease-dependent mesenchymal invasion mode or more recently discover the amoeboid mode that relies on enhanced cell contractility. All modes of cancer cell invasiveness are interconvertible and could be employed by cancer cells in combination. A great deal of effort of the world wide scientific community has been devoted to studying various aspects of cell invasion and migration. However, despite all the effort, the so far incomplete understanding of the plasticity of cancer cells' invasiveness precluded successful development of clinically usable anti-metastatic treatment strategies. The project aims to analyze various aspect of cancer cell invasiveness and in collaboration with 1st Faculty of Medicine to test migrastatic potential of newly developed anti-cancer drugs.

The PGS candidate should have experience in mammalian cell cultivation techniques and basic fluorescence microscopy. Experience with live-cell microscopy and molecular cloning are of further advantage.

Src signaling in regulation of cellular adhesion and mechanotransduction

doc. RNDr. Daniel Rösel, Ph.D.

ID 223021

The project aims to analyze the role of Src-p130Cas-Crk signaling axis in cellular adhesion and mechanotransduction. Within the project the mechanosensory properties of Src and p130Cas will be evaluated taking advantage of in lab prepared Src and p130Cas biosensors. Further, mutagenesis and newly designed specific inhibitors will be used to affect the Src-p130Cas-Crk signaling axis and thus the invasive properties of cancer cells. The PGS candidate should have experience in molecular cloning, mammalian cell cultivation techniques and fluorescence microscopy. Experience with live-cell microscopy, FRET and biophysical methods analyzing mechanical properties of cells are of further advantage.

The determination of p130Cas role in mechanics of Cell-ECM mechanosensing

doc. RNDr. Daniel Rösel, Ph.D.

ID 217751

The ability of cells to sense mechanical properties of surrounding environment is crucial for many physiological as well as pathological processes including morphogenesis, tissue homeostasis or cancer. Cells sense these mechanical cues through specialized mechanosensory proteins. One of such mechanosensory proteins is p130Cas. P130Cas is a major substrate of Src proto-oncogene, plays an important role in oncogenic transformation mediated by the v-crk and v-src oncogenes and increased levels of its human ortholog, BCAR1, are associated with exacerbated prognosis in breast cancer patients. The project aims to determine the mechanistic role of CAS substrate domain in mechanosensing and mechanotransduction and to prepare FRET-based p130Cas-derived biosensors of intracellular mechanical tension.

The PGS candidate should have experience in molecular cloning, mammalian cell cultivation techniques and fluorescence microscopy. Experience with live-cell microscopy, FRET and biophysical methods analyzing mechanical properties of cells are of further advantage.

Mutations in splicing factors linked with retina degeneration - molecular mechanism

doc. Mgr. David Staněk, Ph.D.

ID 221724

Retinitis pigmentosa (RP) is a human hereditary disorder caused by a progressive loss of photoreceptors. Surprisingly, mutations in six key splicing proteins have been associated with autosomal dominant form of RP. The splicing factors are essential for every cell in a human body and thus it is enigmatic why their mutations have such a cell specific phenotype. In our laboratory, we have developed several model systems including mice and eye organoids to study how the RP-linked mutations in splicing factors affect target cell metabolism. The aim of the proposed PhD project is to characterize at molecular level, how RP-linked mutations change behavior of mutated proteins. Specifically, we plan to study how the mutations affect protein and RNA interactome of splicing factors in targeted cells and to identofy small molecules that could revert the observed phenotype.

We seek an enthusiastic colleague with strong interest in RNA biology. Previous experience with molecular biology, cell culture or animal models is welcomed.

Requirements:

• MSc, MRes, Diploma or an equivalent degree in Biology, Biomedicine, Chemistry or related sciences, to be obtained latest by the start of the fall term in September 2020

• Practical experience in the lab working on scientific projects

• Excellent English language skills and the desire to work in a dynamic international team

Biogenesis of spliceosomal snRNPs

doc. Mgr. David Staněk, Ph.D.

ID 223186

RNA splicing is one of the critical steps in gene expression carried out by large complex called the spliceosome. The spliceosome is assembled de novo on each intron to be spliced from basic building blocks named small nuclear ribonucleoprotein particles (snRNPs). The long-term goal of our lab is to determine, how snRNPs and the spliceosome assemble in a complex and chaotic cellular environment. The aim of the project is to describe early steps during snRNP biogenesis and determine molecular details of quality control mechanism that discriminates between mature and immature snRNP complexes. The project involves combination of biochemistry, molecular biology and microscopy approaches to determine factors essential for snRNP maturation and surveillance. Namely, the project focuses on the SMN complex, that is involved in early steps in snRNP biogenesis.

Reviving canonical RNAi in mammals

prof. Mgr. Petr Svoboda, Ph.D.

ID 223254

The aim of the project is to examine different strategies for enhancing mammalian RNA interference (RNAi) in vivo. RNAi is an ancestral antiviral innate immunity pathway abandoned during vertebrate evolution. The project seeks to understand how RNAi could be restored in vivo without significant negative effects and if augmented RNAi could provide broad antiviral defense. The project stems from ERC project D-FENs and will lay foundations for another ERC project aiming on converting knowledge of canonical RNAi and its constraints into a safe antiviral system deployable into infected cells. The PhD project will focus on analysis of effects of augmented RNAi in vivo. Using our gnetically modified mouse models, the aim is to a) examine genetically-induced RNAi pathway activity in different tissues and cell types, b) assess detrimental effects of RNAi in cells, organs and the whole mouse, and c) analyze competition, cooperativity & redundancy between RNAi and mammalian antiviral innate immunity.

Reviving canonical RNAi in mammals

prof. Mgr. Petr Svoboda, Ph.D.

ID 223255

The aim of the project is to examine different strategies for enhancing mammalian RNA interference (RNAi) in vivo. RNAi is an ancestral antiviral innate immunity pathway abandoned during vertebrate evolution. The project seeks to understand how RNAi could be restored in vivo without significant negative effects and if augmented RNAi could provide broad antiviral defense. The project stems from ERC project D-FENs and will lay foundations for another ERC project aiming on converting knowledge of canonical RNAi and its constraints into a safe antiviral system deployable into infected cells. The PhD project will focus on mechanistic understanding of RNAi activation. This will include clarifying the molecular mechanism behind the phenomenon of augmented RNAi and evaluation of strategies for augmenting RNAi for therapeutic use.

Retrotransposon suppression and gene evolution

prof. Mgr. Petr Svoboda, Ph.D.

ID 223256

Retrotransposons are not only parasitic sequences threatening genome integrity but also source of variability and complex changes in gene structure and function. The aim of the project is to analyze evolution of mammalian non-autonomous MaLR retrotransposons and novel adaptations in the mammalian female germline caused by retrotransposon insertions. This work will include analysis of maternal transcriptomes, control of MaLR expression in the germline, and loss of CpG dinucleotides and repressive effects of DNA methylation.

Molekulární a buněčná podstata katarální fáze a transmise pertusové infekce

prof. doc. Ing. Peter Šebo, CSc

ID 213446

Černý, či dávivý kašel, nebo-li pertuse, je významné a mimořádně infekční respirační onemocnění, které před zavedením očkování patřilo k hlavním příčinám kojenecké úmrtnosti. Díky zavedení méně reaktogenních a méně účinných očkovacích látek, se nyní pertuse masivně vrací do nejvyspělejších zemí. Infekce začíná tzv. katarální fází, kdy se bakterie po potlačení imunity hostitele pomnoží v nosohltanu do vysokých počtů a vyvolá rinoreu, tedy nekontrolovatelný výtok z nosu infikovaného jedince, který jej přinutí kašlat a kýchat a tím šířit infekci v aerosolových kapénkách. Molekulární a buněčná podstata tohoto procesu zůstává zcela neprostudovaná a nepoznaná, neb donedávná nebyly k dispozici vhodné zvířecí a buněčné modely k jejímu studiu. Nám se jako prvním povedlo zavést model MyD88 KO myši ve které dochází k šíření infekce B. pertussis z myši na myš. Můžeme proto pomocí mutageneze patogena B. pertussis a pomocí genetické manipulace myší teď začít začít hledat faktory virulence patogena a identifikovat buněčné a molekulární mechanismy, které vedou k otevření těsných spojů mezi buňkami respiračního epitelu, k otevření chloridových kanálů (CFTR) a k nadprodukci mukusu a úniku kapaliny přes epiteliální bariéru, jež vedou k výtoku z nosu infikované myši a transmisi infekce. Zároveň jsme zavedli model polarizovaných epiteliálních vrstev pěstovaných na rozhraní vzduchu a kapaliny, ve kterých lze tyto procesy studovat na buněčné a molekulární úrovni. Cílem projetu bude testovat hypotézu, že součinnost působení pertusového toxinu s adenylátcyklázovým toxinem bakterie B. pertussis a signalizace bakterií-produkovaných TLR ligandů, vede k zvýšení hladiny cAMP v epiteliálních buňkách, zvýšení produkce mucinů pohárkovými buňkami, otevření chloridových kanálů (CFTR), zablokování Rab11-závislého dokování exocystového komplexu do epiteliální membrány tvořící těsné mezibuněčné spoje a k narušení integrity epiteliální bariéry. Ke studiu komplexu těchto dějů budou využity jak zvířecí modely, tak techniky molekulární biologie a genetiky buněčné biologie a fluorescenční mikroskopie ve vysokém rozlišení pro analýzu buněčných pochodů v infikovaných epiteliálních vrstvách. Cílem bude objasnit, proč nám teče z nosu, když se nám v něm usadí původce černého kašle.

Chromosome dynamics and integrity in mammalian oocytes and early embryos

doc. RNDr. Petr Šolc, Ph.D.

ID 223205

Correct chromosome segregation during oocyte meiosis and first cell divisions of embryos is critical for reproduction and healthy offspring. Defective chromosome segregation (aneuploidy) in oocytes and embryos leads to developmental defects (e.g., Down syndrome) or infertility. Spindle apparatus is an essential molecular machine ensuring normal chromosome segregation. In somatic mitotic cells, bipolar spindle formation is supported by two centrioles containing centrosomes. However, in oocytes, multiple acentriolar microtubule organizing centers (MTOCs) facilitate spindle formation, and MTOCs are finally sorted into the two spindle poles. We have identified signaling of three Aurora kinases (Aurora A, B, C) as crucial for effective spindle formation and normal chromosome segregation in mammalian oocytes. Now we would like to discover how these kinases cooperate and also with chromosome associated RanGTP signaling during spindle formation and chromosome segregation in mammalian oocytes. Although early embryos are dividing by mitosis, their spindles are still meiotic, and they contain MTOCs rather than centrosomes. During preimplantation development from the zygote (fertilized egg) to the blastocyst, the spindle gradually shifts from the meiotic to the mitotic form. Only in blastocyst cell division is supported by true mitotic spindle containing two classical centrosomes. Now, we would like to uncover how Aurora kinases participate in this step by step development from meiotic to mitotic spindles.

Recently it was shown that not only whole chromosome missegregation (aneuploidy) but also increased double-strand DNA breaks in oocytes and embryos could account for infertility or health problems of offspring. Surprisingly, we have found that oocytes do not have DNA damage checkpoints preventing cell cycle progression in the presence of increased DSBs. On the other hand, we have discovered that DSBs repair machinery works during meiotic maturation on already condensed chromosomes what is in strong contrast to somatic cells where DNA repair is suppressed in mitosis. Very interestingly, our recent data suggest that long non-coding RNAs may be involved in chromosome integrity and possibly DNA repair in oocytes. Now, we would like to uncover how does the DSBs repair work in this very important moment of our development.

We are using mouse genetic tools, combined with chemical biology approaches and advanced live-cell imaging (both confocal and light-sheet microscopy) and computer image analysis to uncover how chromosome segregation and integrity are ensured in mammalian oocytes and embryos. This project will bring important knowledge for human reproductive medicine, and we plan that at the right moment, we will move to human oocytes to see how does it work?

Further reading:

Drutovic D, Duan X, Li R, Kalab P,Solc P. RanGTP and importin β regulate meiosis I spindle assembly and function in mouse oocytes. EMBO J. 2020 Jan 2;39(1):e101689.

Nguyen AL, Drutovic D, Vazquez BN, El Yakoubi W, Gentilello AS, Malumbres M,Solc P, Schindler K. Genetic Interactions between the Aurora Kinases Reveal New Requirements for AURKB and AURKC during Oocyte Meiosis. Curr Biol. 2018 Nov 5;28(21):3458-3468.e5.

Balboula AZ, Nguyen AL, Gentilello AS, Quartuccio SM, Drutovic D,Solc P, Schindler K. Haspin kinase regulates microtubule-organizing center clustering and stability through Aurora kinase C in mouse oocytes. J Cell Sci. 2016 Oct 1;129(19):3648-3660.

Mayer A, Baran V, Sakakibara Y, Brzakova A, Ferencova I, Motlik J, Kitajima TS, Schultz RM,Solc P. DNA damage response during mouse oocyte maturation. Cell Cycle. 2016;15(4):546-58.

Evolutionary dynamics of chromosomal rearrangements and identification of sex chromosomes within the family Pipidae

RNDr. Tereza Tlapáková, Ph.D.

ID 223187

The family Pipidae represents a unique group of ancient and evolutionary conserved frogs. Due to a geographical distribution it is divided into the subfamily Pipinae with the Neotropical genus Pipa and subfamily Xenopodinae encompassing the most African species of subgenera Xenopus and Silurana. The project is focused on the study of chromosomal evolution and identification of sex determining region among mentioned genera employing FISH-TSA and Zoo-FISH and SNPs genotyping of Illumina RADseq and HiSeq sequences. Hybridization of X. tropicalis and Pipa whole-chromosomal painting probes with karyotypes of each subfamily representatives will definitively shed a new light on the evolutionary relationships among studied species. Identification of sex-linked SNPs allows the physical mapping of sex determining region in analyzed species and will contribute to the better understanding of a sex determining mechanisms based on the chromosomal rearrangements after polyploidization event.

The main aim of this project is a comparative cytogenetic and genomic analysis of geographically isolated groups of the family Pipidae encompassing four genera: Pipa, Hymenochirus, Silurana and Xenopus. Disentangling their mutual evolutionary topology via study of chromosomal rearrangements will be performed using chromosome banding, NOR detection by FISH with ribosomal gene probes and advanced cytogenetic techniques - painting FISH and Zoo-FISH employing the WCPs prepared by the laser microdissection of separately isolated S. tropicalis and Pipa sp. chromosomes. FISH-TSA of approx. one hundred Pipa cDNA probes would allow the construction of physical map and identification of inversion and/or translocation events between African and Neotropical representatives of the family Pipidae.

Construction of the mammalian primary cilium

Mgr. Vladimír Varga, Ph.D.

ID 223227

The primary cilium is an important organelle with signaling and sensory roles. The ciliary cytoskeleton is formed at its distal end, but little is known about the process and about proteins orchestrating it. Based on work on our model system, an experimentally highly tractable parasite Trypanosoma brucei, we recently identified novel mammalian proteins localizing to the distal end of the cilium. In the proposed project the function of these proteins will be studied. In particular, the possible role of the proteins in establishing the pattern of microtubules of different lengths in the cilium will be addressed by live cell imaging of wild type and mutant cell lines. Advanced electron microscopy techniques will also be employed. In addition, murine knock-out models will be characterized. We look for a highly motivated student eager to learn new techniques and eventually to drive the project.

Flagellum distal end-localizing proteins of the human parasite Trypanosoma brucei

Mgr. Vladimír Varga, Ph.D.

ID 223225

The flagellum is an organelle essential for cell motility with important signaling and sensory roles. Using an experimentally highly tractable protozoan Trypanosoma brucei, a causative agent of African sleeping sickness, we recently identified a number of flagellum distal end-localizing proteins involved in construction of the organelle, regulation of its motility and in cell morphogenesis. In the proposed project trypanosome cell lines mutant in the proteins will be characterized in detail. Advanced electron microscopy techniques will be employed to reveal their localization into particular flagellar structures. Biochemical approaches will be employed to gain a mechanistic insight into activities of the proteins. We look for a highly motivated student eager to learn new techniques and eventually to drive the project.

Targeting hematological malignancies using fragment-based drug design

Ing. Václav Veverka, Ph.D.

ID 212077

The project will be focused on the knowledge-based design of active compounds targeting several proteins or protein-protein interactions, which are implicated in the development of hematological malignancies. The compounds will be developed using the fragment-based approach. The work on this project provides the opportunity to acquire an expertise in protein biochemistry, biophysics and structural biology, as well as to get an insight into the rational drug design process through a close collaboration with the medicinal and computational chemists.

Transient nuclear protein-protein interaction networks

Ing. Václav Veverka, Ph.D.

212076

Many processes implicated in the regulation of DNA transcription are driven by highly modular protein-protein interaction networks that are not exclusively maintained by the well structured protein domains but also by highly dynamic regions of interacting proteins. The aim of the doctoral project will be an identification as well as investigation of the physiological and pathological roles of these transient interactions using highly interdisciplinary approach, including protein biochemistry, biophysics, cell and structural biology.

The role of perinuclear actin stress fibers in the cell polarization and migration

Ing. Tomáš Vomastek, Ph.D.

ID 212177

In adherent cells actin cytoskeleton forms morphologically and functionally distinct structures including parallel actin bundles in filopodia, branched polymerizing actin network in lamellipodium and ventral contractile stress fibers. In addition to these actin structures that are confined to the cell's basal side, cells also possess so called perinuclear actin cap fibers that rise from the ventral side of the cell above the nucleus and mechanically link focal adhesions with the nuclear envelope. Although perinuclear actin fibers are typical for migrating cells their functions in the cell migration remain largely unknown.

The proposed topic of PhD thesis focuses on the role of perinuclear actin fibers in cell migration and invasion. These fibers may stimulate nuclear movement during the establishment of front-back migratory polarity and, consequently, promote cell migration. We aim to determine the mechanisms that control the assembly of perinuclear fibers and how are these mechanisms employed to facilitate nuclear movement and cell migration. It will be also determined whether perinuclear fibers contribute to tumor cell invasiveness and metastasis.

The methodology will include the work with mammalian cell culture, gene editing using CRISPR/Cas9 system, RNA interference, and life-cell and immunofluorescence microscopy including superresolution microscopy. Standard molecular biology techniques such as DNA cloning, protein expression, SDS-PAGE and western blotting will be also utilized.

In the footsteps of the RACK1 role in cytokinesis

Ing. Tomáš Vomastek, Ph.D.

ID 223079

RACK1 (The Receptor of Activated C Kinase 1) is an evolutionary conserved scaffold protein participating in pleiotropy of cellular processes. As such, RACK1 is also involved in the cell cycle regulation. In our lab, we found that partial depletion of this essential protein leads to morphological aberrations and a defect in cell-cell separation. The aim of proposed PhD project is to elucidate a role of RACK1 in these processes.

The project will be conducted within the Institute of Microbiology of the CAS in Krc and it is currently supported by Czech Science Foundation (GACR 19-07603Y) and Czech BioImaging infrastructure (MEYS CR, LM2018129).

For more information, contact the project supervisor: Tomas Grousl

tomas.grousl@biomed.cas.cz

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The role of the ERK signaling pathway in in the control of epithelial cell proliferation and differentiation

Ing. Tomáš Vomastek, Ph.D.

ID 212178

The ERK pathway is an evolutionary conserved signaling cascade that is activated by a multitude of extracellular signal and in turn converts these signals to diverse biological outcomes such as changes in gene expression, cell proliferation, differentiation, apoptosis or cell migration. The ERK signaling cascade displays a three tier architecture consisting of protein kinases, Raf, MEK and ERK, and the signal is transmitted sequentially from Raf to MEK to ERK. Active ERK phosphorylates and thus alters the function of a diversity of cellular proteins ultimately bringing about the changes leading to responses appropriate for the particular extracellular signal. Importantly, altered regulation of ERK signaling due to Ras or B-Raf activating mutations is central to cancer development and cancer progression where it promotes the expression of pro-oncogenic genes, uncontrolled proliferation, cell invasion and metastasis formation.

The proposed topic of PhD thesis aims to investigate changes that are induced in model epithelial cell lines by constitutive activation of the ERK pathway. The role of the ERK pathway will be also examined in the context of the development of Head and Neck cancer (HNSCC), where we will analyze patient tumor samples and the occurrence of genomic mutations.

The methodology will include the work with mammalian cell culture, gene editing using CRISPR/Cas9 system, RNA interference, life-cell and immunofluorescence microscopy including superresolution microscopy. Standard molecular biology techniques such as DNA cloning, protein expression, SDS-PAGE and western blotting will be also utilized.

A role of Stress granules in the modulation of cell signallization upon stress conditions

Ing. Tomáš Vomastek, Ph.D.

ID 212181

The field of translation Stress granules (SGs) is currently in the enormous interest of scientific community due to many reasons. First of all, SGs are linked to the most devastating diseases of these days, cancer and neurodegenerative disorders. Second, SGs are being formed by so called “phase separation” process, which we do not know much about yet. Phase separation is responsible for an assembly of subcellular membrane-less organelles recently getting more and more attention of researchers. Last, but not least, despite SGs were identified decades ago, their role in cellular metabolism is still not completely elucidated.

RACK1 (The Receptor for Activated C Kinase 1) is evolutionary conserved multifunctional scaffolding protein. It’s role in cellular signalling pathways is investigated from many different points of view. Among other, RACK1 is also a component of SGs. The goal of the PhD project is to elucidate a role of this protein in SGs dynamics and related cellular processes, i.e. translation regulation and stress response, at mammalian cell culture model systems.

The host lab of Cell Signalization is a part of the Institute of Microbiology of CAS, located in Krč campus. We offer a possibility to work on actual scientific tasks using state-of-the-art molecular and cellular biology methods (e.g. super-resolution microscopy, CRISPR/Cas9, optogenetics tools, etc.). In addition, a contract will be concluded with the PhD student and he/she is going to be paid beyond his/her scholarship. On the other hand, we expect good working practise in the lab, the flexibility and the willingness to learn new methods.

Work will be performed under supervision of T. Grousl, PhD. (tomas.grousl@biomed.cas.cz; +420 723 521 611)

A role of Stress granules in the modulation of cell signallization upon stress conditions

Ing. Tomáš Vomastek, Ph.D.

ID 211488

The field of translation Stress granules (SGs) is currently in the enormous interest of scientific community due to many reasons. First of all, SGs are linked to the most devastating diseases of these days, cancer and neurodegenerative disorders. Second, SGs are being formed by so called “phase separation” process, which we do not know much about yet. Phase separation is responsible for an assembly of subcellular membrane-less organelles recently getting more and more attention of researchers. Last, but not least, despite SGs were identified decades ago, their role in cellular metabolism is still not completely elucidated.

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Work will be performed under supervision of T. Grousl, PhD.

tomas.grousl@biomed.cas.cz; +420 723 521 611

Effects of FGF21 on adipose tissue metabolism

RNDr. Petr Zouhar, Ph.D.

ID 221782

FGF21 (Fibroblast growth factor 21) is a hormone secreted mainly from liver in response to stimuli such as fasting and ketogenic diet. Although its main physiological role is still a contested topic, FGF21 has become a promising therapeutic target for obesity and Type 2 diabetes mellitus due to its powerful body weight-lowering and insulin-sensitizing effects. The main aim of the PhD project will be detailed characterization of FGF21 effects in adipose tissue of model animals, namely changes in gene expression and rate of lipolysis, de novo lipogenesis and fatty acid re-esterification. Since these metabolic processes are known to be linked to preservation of healthy status of the tissue, their potential regulation by FGF21 could thus represent an important part of the mechanism of overall beneficial action of FGF21.Basic PhD scholarship will be supplemented by salary covered by grants of the Department.