

**Expression of Huntingtons disease markers in eye tissues of animals transgenic or knock-in for the mutated huntingtin**

MUDr. Taras Ardan, Ph.D.

ID 212992

Huntingtons disease (HD) is a genetic neurodegenerative disorder that affects muscle coordination (chorea) and leads to cognitive decline and psychiatric problems (dementia). It typically becomes noticeable in mid-adult life. This disease is caused by an expansion of a polyglutamine (polyQ) domain in the protein of huntingtin (htt). PolyQ expansion above 35–40 results in the disease associated with htt aggregation into inclusion bodies. The recent studies have shown that the illness arises not only in neurons, but also in non-neuronal cells. Purpose of this study will be to investigate the expression of selected HD markers (on htt and their aggregates into nuclear inclusions, micro- and microgliosis, proteolytic enzymes) in eye tissues (retina, corneas, conjunctivas) of minipig transgenic for the mutated huntingtin and compare them with tissues of wild type (WT) minipig. Since mutant Htt (mtHtt) and especially their small proteolytic fragments are very toxic to all HD cells (particularly those of neuroectodermal origin such as neurons or retinal pigment epithelial cells), it has been suggested that upregulated proteolysis of mtHtt plays a crucial role in the HD pathogenesis including damage of the retina.

## **The role of metalloproteases in the damaged cornea**

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Severe corneal damage evokes very strong inflammatory response which is characterized by infiltration of inflammatory cells and massive production of proteolytic enzymes and proinflammatory cytokines. The metalloproteases (MMPs, ADAMs) are intracellular and extracellular proteases that involve in remodeling the extracellular matrix, and as major ectodomain sheddases, release the a variety of cell surface proteins, including growth factors, cytokines, cell adhesion molecules and receptors. By cleaving extracellular matrix components and releasing proinflammatory mediators, metalloproteases can have profound effects on development of excessive inflammatory reaction. Up to now, the role of metalloproteases in corneal injury and healing has not been fully established. Hence, the aim of this project is to contribute to the better elucidation of the function of these enzymes in seriously damaged cornea and after its therapy by stem cells. Because activities of metalloproteases are regulated by their tissue inhibitors, a further aim of the project is to investigate some specific inhibitors of metalloproteases. Aim: To investigate the proteolytical enzymes belonging to the group of metalloproteases in human diseased corneas and on a model of experimentally damaged cornea of a laboratory animal in normal, injured and stem cells-treated cornea.

## **Mechanisms of cell reprogramming**

RNDr. Petr Bartůněk, CSc.

ID 212243

The project will focus on mechanisms of epigenetic control of hematopoietic cell reprogramming using unique model of zebrafish erythroid progenitor cell line that can be reprogrammed into myeloid lineage using cytokine switch only. The candidate will employ already established methods like ATAC-seq, ChIP-seq, RNAseq and further develop single-cell-based technologies.

## **Characterization of $\gamma$ -tubulin filaments and nuclear functions of $\gamma$ -tubulin**

doc. RNDr. Pavla Binarová, CSc., DrSc.

ID 212399

Besides its well-defined role in microtubule nucleation there are less understood functions of  $\gamma$ -tubulin in nuclear processes such as repair, transcription, telomeres positioning as well as functions in the cell cycle regulation. We found recently that plant  $\gamma$ -tubulin preserves intrinsic ability of prokaryotic tubulins to polymerize filaments and that ability to polymerize is conserved for animal  $\gamma$ -tubulin. Structure of  $\gamma$ -tubulin filaments and extent of lateral interaction in formation of higher order assemblies will be studied further using TEM/cryoTEM and super-resolution microscopy with focusing to differences between human and plant  $\gamma$ -tubulin. Uncovering how formation of  $\gamma$ -tubulin fibrils is spatially and temporally regulated will help understanding role of  $\gamma$ -tubulin in nuclear processes. We identified DNA repair proteins Rad51, BRCA, and RETINOBLASTOMA RELATED, and E2F transcription factors to interact with  $\gamma$ -tubulin. Chromatin immunoprecipitation will be used to uncover DNA targets of  $\gamma$ -tubulin. ChIP data together with data on protein interactions of  $\gamma$ -tubulin in the nuclei under DNA stress will help to get insight into its role in genome maintenance. Proposed research will contribute to expanding field understanding of cellular functions of filament-forming  $\gamma$ -tubulin.

## **Regulation of cancer cell invasiveness by stress-associated signaling**

Doc. RNDr. Jan Brábek, Ph.D.

Only for students enrolled in STARS program! During its life, the cell is exposed to a number of stimuli that indicate a possible threat to its integrity. These stimuli trigger activation of the stress signaling pathways that control the adaptation of the cell to the stimulus or lead to apoptosis. MAP kinases p38 and JNK play a key role in stress signaling, triggering a phosphorylation cascade to regulate effector protein activity in response to extracellular signals. MAPK pathways generally regulate many cellular processes in addition to stress response, such as cell proliferation, differentiation or secretion of various factors. Their role in carcinogenesis is thus not surprising. While their effect on cellular transformation is relatively well studied, less is known about how they regulate the metastasis process, which, however, is responsible for most of cancer deaths. During the invasion of the extracellular space cancer cells use various migration modes, most commonly the collective, amoeboid or mesenchymal mode of migration. The fact that these modes are often interchangeable complicates successful treatment. The influence of MAP kinase pathways on cellular invasiveness has been extensively studied, however, whether they participate in the selection of migration mode has not yet been analyzed. The ability of cancer cells to switch between invasion modes underlies the metastatic disease. Our preliminary results indicate the key role of MAP kinase signaling pathways during individual migration strategies. The aim of this project is to find out how the components of stress-associated MAP kinase signaling pathways contribute to cancer cell invasiveness and how they synergize to drive the metastatic behavior of cancer cells.

## **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.

## **Role of mTORC1 in the processing of the amyloid protein associated with Alzheimer's disease.**

Mgr. Marek Cebecauer, Ph.D.

ID 212599

Alzheimer's disease (AD) is the most frequent neurodegenerative disease and might result in personality changes. Deposition of extracellular amyloid (A $\beta$ ) plaques and interneuronal tangles composed of tau protein are considered as the main molecular symptoms of AD. A $\beta$  is a product of the intracellular proteolytic pathway of amyloid precursor protein (APP). The precise localization of APP cleavage products is poorly understood. mTORC1 is a key component of the signalling pathway which regulates autophagy, lipid synthesis, transcription, translation and cell growth. mTORC1 hyperactivity has been demonstrated in AD model organisms but also in post-mortem human AD brains. Altered mTORC1 activity has an impact on cognitive abilities of AD mice, as well as on A $\beta$  and tau pathology. The aim of this project is to focus on the link between mTORC1 and APP processing in neuroblastoma cells. We will use the state-of-the-art super-resolution microscopy to investigate the localization of APP and its proteolytic products in neuronal cells and to determine its co-localization with mTORC1. Cholesterol molecules influence AD pathology either by affecting APP processing or by interfering with A $\beta$  aggregation. We aim to investigate the cooperative impact of mTOR signalling and varying cholesterol levels on the APP processing.

The student will use recombinant DNA techniques to generate variants of APP with fluorescent proteins and other functional tags, immunofluorescence for specific labelling of molecular structures and a large panel of microscopy techniques to visualise APP processing and mTOR1 impact in cultured neurons. No specific skills are required in advance except of the enthusiasm for new discoveries.

## **Regulation of surface receptors by membrane nano-topology in lymphocytes**

Mgr. Marek Cebecauer, Ph.D.

ID 212224

CD4<sup>+</sup> T cells co-ordinate immune responses against infections and cell transformations. The antigen receptors (TCRs) trigger signals to activate T cells but a handful of surface receptors (e.g. CD2, CD4/8, CD6 and PD-1) can regulate the output of these processes. Molecular mechanisms co-ordinating the action of these receptors remain incompletely understood, especially, the spatio-temporal organisation of the receptors and associated signalling events. 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 nanoscopic topological organisation of receptors on T cells before and during recognition of antigens. Based on these results, we are currently studying previously overlooked, TCR-independent role of CD4 in T cell activation and homeostasis. However, the activity of TCRs and CD4 can be modulated by other surface receptors, topological organisation of which is unknown. We plan to map distribution of CD2, CD5, CD6 and PD-1 on the surface of T cells and characterise molecular processes that regulate activity of these cells in time and space (3D, nanoscopic). The project will include the use of the state-of-the-art super-resolution microscopy (co-developed in our laboratory), functional imaging of living T cells and recombinant DNA technologies.



## **Exploring mechanisms of DNA replication restart upon collisions between transcription and replication complexes**

RNDr. Jana Dobrovolná, Ph.D.

ID 211798

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.

## **Nanoparticle-assisted targeted in vivo delivery of ABCA4 gene for Stargardt dystrophy**

Ing. Zdenka Ellederová Ph.D.

ID 212198

Stargardt's disease (STGD1) is a blind disease, which is associated with ABCA4 mutation with no curative treatments. The ABCA4 cDNA is 6.8 kb and thus it's too large for the AAV vectors, used successful for delivery of therapeutic genes. Nanoparticles (NPs) gained importance for gene delivery that may successfully target genetic diseases, particularly those associated with large genes. Here, we aim to develop a non-viral delivery system using NPs for the delivery of ABCA4 therapeutic gene and mediate improvement in STGD1. First, we will generate poly (d,l lactide-co-glycolic acid) (PLGA) and human serum albumin (HSA) NPs to test their nuclear transfer in a RPE cells. Second, we will develop PLGA and HSA NPs carrying the plasmid expressing ABCA4. The delivery efficiency in ABCA4 deficient cells and in Abca4-deficient mice injected subretinally will be tested. Subsequently, we will use the data from this project in subsequent preclinical tests in large animal models such as the pig, the project contributes to the development of safe gene therapy approaches for STGD1.

## **Phenotypic Studies of the Huntington's Disease Knock –in Minipig Model**

Ing. Zdenka Ellederová Ph.D.

ID 212197

Recent promising treatments for Huntington's disease (HD) may require pre-clinical testing in large animals. We aim to characterize the new knock in (86Q KI-HD) minipig model and identify pre-manifest biomarkers. The non-invasive approach will include behavioral and motoric testing, periodical collection of blood, cerebrospinal fluid (CSF), and fibroblasts. Mutated huntingtin, neurofilament light protein, a panel of cytokines, different markers of metabolism and mitochondria biogenesis as well as standard hematology and biochemistry tests will be measured in blood, CSF or both. Furthermore, animals will be sacrificed at 6, 12, and 24 months of age in order to perform biochemical and histological analysis of tissues. We will examine 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 myelination. We will also address 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.

## **The role of phosphoinositides in spatiotemporal regulation of nuclear processes.**

prof. RNDr. Pavel Hozák, DrSc.

ID 211746

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.

## **Functions of lamin A - phosphoinositides complexes in the cell nucleus**

prof. RNDr. Pavel Hozák, DrSc.

ID 211743

Lamins are intermediate filament proteins involved in a variety of nuclear functions, such as regulation of gene expression, DNA replication, DNA repair, chromatin organization, and cellular signaling. Mutations in lamins cause severe diseases – laminopathies. Lamins in the cell nucleus exist in two distinct pools – in nuclear lamina and in the nuclear interior. While much less is known about the nucleoplasmic lamins, their functional specificity and importance in gene expression has been increasingly recognized. Our preliminary data demonstrate that lamin A forms a range of complexes with phosphatidylinositol 4,5-bisphosphate exhibiting different mechanism of binding and different composition. This project focuses on detailed characterization of these complexes by biochemical, structural and advanced microscopy methods using various experimental models. The project will implement molecular biology and biochemistry methods as well as the state-of-the-art imaging techniques including fluorescence, confocal and super-resolution (SIM, STED, STORM) microscopy. The project is supported by recently awarded funding from the Grant Agency of the Czech Republic

## **Exploring the role of vinculin/DEB-1 in eukaryotic meiosis**

prof. RNDr. Pavel Hozák, DrSc.

ID 211755

Our department focuses on the vinculin/DEB-1 protein, and we originally confirmed it in the nucleus of meiotic cells in *Mus musculus* and *C. elegans*. Our goal is to study his role in the gametogenesis of this two eukaryotic model organisms.

We study vinculin in mouse spermatogenesis and oogenesis using model strains of vinculin conditional knock-out that we developed in our laboratory. Specifically, we proved that vinculin is important for homologous chromosome pairing and formation of the synaptonemal complex, possibly through the vicnulin involvement in the ubiquitine-proteasome system. In *C. elegans* we showed the interaction of the DEB-1 (ortholog of mammalian vinculin) with the SUN/KASH module at the nuclear periphery. DEB-1 depletion caused a defect in the stabilization of the chromosomal pairing centers and subsequent aneuploidy.

The PhD project will implement molecular biology and biochemistry methods as well as the state-of-the-art imaging techniques including fluorescence, confocal and super-resolution (SIM, STED) microscopy (SIM figure in the heading, such a detailed picture of synaptonemal complex would not be possible few years ago). The project is supported by funding from the Grant Agency of the Czech Republic.

Candidates should possess M.Sc. (Mgr.) degree or equivalent in molecular/cellular biology or biochemistry, good English, independent thinking, strong interest in basic research and experimental work, dedication to learn and develop new techniques.

## **Design and biochemical analysis of proteins / protein libraries incorporating unnatural amino acids**

Mgr. Klára Hloučová, Ph.D.

ID 213245

One of the biggest puzzles in protein evolution is the selection of its amino acid alphabet. Only about half of the canonical alphabet was available prebiotically while the evolutionary “late” amino acids developed through biosynthetic pathways or early life. At the same time, many other alpha-amino acids (that are not part of today’s protein alphabet) were available to use.

This project aims to explore the structure/function-forming potential of the proteinogenic and non-canonical yet prebiotically abundant amino acids (such as aminobutyric and diaminobutyric acid). An algorithm used in our group for design of random sequence protein libraries will be modified to design sequences incorporating unnatural amino acids, using codon reassignment. Commercial and home-made cell-free protein translation systems will be modified in collaboration with our international partners to allow for synthesis of such proteins and protein libraries. Finally, the structural and functional properties of such proteins will be analysed in our group.

The results of this project will provide structural and functional consequences of the most prebiotically abundant non-canonical amino acids and help elucidate the protein structure/function factors in the transitions of protein alphabet evolution.

## **Solving the principals of dental initiation, activation and inhibition / Řešení principů iniciace zubů, jejich aktivace a inhibice**

RNDr. Mária Hovořáková, Ph.D.

ID 212072

Development of the tooth, similarly to other ectodermal derivatives, is running based on two embryonic structures: from neural crest and ectodermal placodes. The mouse is an optimal model for studying induction and formation of tooth germs with different developmental fates (rudimentary and functional). Dental placodes are presented in the prospective incisor and molar areas of the lower and upper jaws in the mouse embryo, and specific genes initiating tooth budding are expressed there. How these areas are defined is not known.

The aim of this study will be to search for molecules responsible for interaction epithelium-mesenchyme in the areas of prospective odontogenesis, considering the tooth type and its function. In vitro cultures will be used, allowing us to use activators, inhibitors or proteins added in the medium or using beads for local application. We will track possible formation of dental placode in nondental regions – for example in prospective diastema. For the detection of markers presence in target regions we will use in situ hybridisation and immunohistochemistry.



## **Discovery and characterization of novel tumor suppressor pathways**

Mgr. Zuzana Kečkéšová

ID 213387

Cancer has been affecting people since the beginning of our history. Despite considerable efforts in the last decades to find a cure, cancer-related death remains one of the leading causes of death worldwide. Our previous work, aimed at identifying novel tumour suppressors, allowed us to identify Lactamase-B-like (LACTB) protein, a novel mitochondrial tumour suppressor that acts through modulating mitochondrial lipid metabolism and differentiation of cancer cells. In this project we will build on this study by examining the mechanistic processes underlying this pathway. We will examine in more detail the interconnection between mitochondrial lipid metabolism and cancer cell differentiation, the physiological regulation of this pathway and we will identify the substrate of LACTB enzyme. This will uncover new vulnerabilities of cancer cells; the knowledge of which can help us in our battle against cancer.

## **Evolution of Cell Types**

RNDr. Zbyněk Kozmík, CSc.

ID 212628

Cephalochordate amphioxus serves as a proxy to ancestral chordates. Although amphioxus lacks the specializations and innovations of vertebrates, it shares with them a basic body plan and has multiple organs and structures homologous to those of vertebrates. For these reasons, amphioxus has widely been used as a reference outgroup to infer ancestral versus novel features during vertebrate evolution (Marletaz et al., 2018). Over the past few years amphioxus has become an established laboratory model and its cultures can be maintained throughout the year at the Institute of Molecular Genetics. This allows for an implementation of plethora of molecular and genetics approaches common to classical vertebrate models such as mouse, chick or fish. Project will focus on evolution of cell types, ancestral chordate features and vertebrate-specific innovations, using comparative analysis between amphioxus and zebrafish. The methods used will include scRNA-seq, bioinformatics, gene knockouts using CRISPR/Cas9 system, gene expression studies by whole-mount in situ hybridization and reporter gene transgenesis.

## **Developmental genetics of amphioxus: a window into the evolution of vertebrate gene regulation**

RNDr. Zbyněk Kozmík, CSc.

ID 211235

during vertebrate evolution. Over the past few years amphioxus has become an established laboratory model and its cultures can be maintained throughout the year at the Institute of Molecular Genetics.

Recent publication on Amphioxus functional genomics and the origins of vertebrate gene regulation (Marlétaz et al., 2018) provides huge genomic resource for future studies focused on gene regulatory mechanisms. Project will focus on function of enhancers in gene regulation, molecular dissection of enhancer activity and enhancer evolution. Special attention will be paid to the question of how the regulatory information encoded in the invertebrate (amphioxus) enhancer becomes interpreted in the context of heterologous vertebrate species (fish). The methods used will include bioinformatics, gene isolation, gene knockouts using CRISPR/Cas9 system, gene expression studies by whole-mount in situ hybridization and immunohistochemistry, enhancer identification and isolation, enhancer knockouts in the context of the entire organism using CRISPR/Cas9 system and reporter gene transgenesis.

References:

Marlétaz et al., 2018

Amphioxus functional genomics and the origins of vertebrate gene regulation.

Nature. 2018 Dec;564(7734):64-70. doi: 10.1038/s41586-018-0734-6. Epub 2018 Nov 21.

## **In vitro study of human uric acid transportome**

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

ID 212187

Uric acid is a primary metabolite of purine degradation in human. It is an important antioxidating substrate and supposed blood pressure regulator. Uric acid is reabsorbed in kidney via epithelial cells of proximal tubule. The most common allelic variants of uric acid transporters (URAT1, GLUT9, ABCG2, OAT1, OAT3 and MRP4) were described in human by genome-wide association studies (GWAS). So far, only functional analysis based on the transport efficiency or kinetics of individual allelic variants were performed. However their mutual interactions in in vitro condition using cell culture were not determined yet..

The aim of this project is the study of human uric acid transportome using human kidney epithelial cell culture and CRISPR/Cas9 and siRNA approach to knock-out or downregulate particular uric acid transporters or their allelic variants. On the protein level specific inhibitors of particulate transporters would be employed. The main scientific questions is: “how downregulation or knock-out of selected wt uric acid transporter(s) or their allelic variants affects the uric acid uptake or efflux by epithelial cells via remaining proteins”. To address this question we would determine the transport of radioactive labeled uric acid in apical and basolateral part, perform the kinetic studies and functional studies employing *Xenopus laevis* oocytes.

## **Proteomic characterization of extracellular vesicles in Huntington's disease**

Mgr. Helena Kupcová Skalníková, Ph.D.

ID 212115

Huntington's disease is a rare hereditary neurodegenerative disorder caused by expanded CAG repeats in gene for protein huntingtin. Mutated huntingtin protein (mHTT) undergoes cleavage by proteases. Resulting mHTT fragments accumulate and are toxic for cells, predominantly for medium spiny neurons in the striatum. Gene therapy using AAV vectors was currently developed to lower mHTT expression in brain. However, there is lack of biomarkers to study disease progression or monitoring of therapy effects. Extracellular vesicles (EVs) are membrane enveloped particles released from cells to surrounding environment and body fluids. As the EVs carry proteins and RNAs of the source cells, they represent potential source of disease biomarkers. The aim of this project is proteomic characterization of EVs released to body fluids of experimental minipig model of Huntington's disease as well as to blood plasma of the human patients or supernatants of human in vitro cultured cells. Special attention will be paid on study of huntingtin forms in EVs in correlation to phenotype.

Methods: biochemical techniques, western blotting, electron microscopy, mass spectrometry, bioinformatics; might be involved: immunological methods (Luminex, ELISA), flow cytometry, cell culture or others.

## **Intercellular relations in tumour stroma and their effects on tumour expansion**

Mgr. Helena Kupcová Skalníková, Ph.D.

ID 212116

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 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 characterization of secreted factors in microenvironment of melanoma and their effects on tumour growth and prognosis in porcine MeLiM model of melanoma and human cells.

Methods: biochemical techniques, immunological methods (Luminex, ELISA), proteomics (mass spectrometry, bioinformatics); might be involved: surgery on animals, cell culture, flow cytometry, isolation and characterization of extracellular vesicles.

## **Flt-3 kinase and its mutations in the acute myeloid leukemia**

RNDr. Kateřina Kuželová, Ph.D.

ID 211980

The acute myeloid leukemia (AML) is an aggressive malignant disease with frequent relapses and relatively poor outcome. The heterogeneity of leukemogenic mechanisms is the main source of leukemia stem cell resistance to therapy, which often prevents complete eradication of the disease. Gain-of-function mutations in the receptor tyrosine kinase Flt-3 belong to the most frequent recurrent mutations in AML, and are associated with worse prognosis. In the majority of cases, increased Flt-3 activity in AML is due to internal tandem duplications (ITD) within the autoregulatory domain. Alternatively, mutations can occur in Flt-3 kinase domain (TKD). It was shown recently, that the impact of both mutation types on AML prognosis is not identical and differences in activated signalling pathways have been found. The molecular mechanisms of leukemogenesis and therapy resistance associated with Flt-3 mutations are only partly elucidated. Flt-3 activity plays a role in hematopoietic cell development, proliferation and survival. It is important for dendritic cell maturation, thereby affecting the immune system function. In addition, Flt-3 involvement in other processes, e.g. cell adhesion, was reported. Whereas cell lines expressing Flt-3-ITD are available, no model cell line with Flt3-TKD was established so far. The aim of this project is to create a model system allowing for comparison of Flt3-ITD with Flt3-TKD on the same genetic background, and to analyse possible differences in functional consequences of these mutations. The methods employed will include namely molecular cloning, proteomics (mass spectrometry), flow cytometry, analysis of the cell metabolism, and of the cell adhesion. Some experiments will be performed on primary samples from leukemia patients (e.g. analysis of Flt3 surface expression, possible correlation of Flt-3 mutation with the amount of regulatory T-cells).

## **Molecular mechanisms of microtubule-based intracellular transport**

RNDr. Zdeněk Lánský, Ph.D.

ID 212061

The generation of directed movement in cells underpins a number of essential cellular processes, as for example the trafficking of organelles or movement of chromosomes during cell division. Although many proteins involved in these processes have been identified, the molecular mechanisms of how these proteins act collectively to drive the directed movement remain to a large extent unclear.

In this project we will focus on some of the following microtubule-based transport processes, namely generation of movement via i) the action of microtubule-associated molecular motors, such as kinesin-1, ii) non-motor diffusible microtubule-associated cross-linkers, such as the member of the Ase1/PRC1/MAP65 family and iii) the dynamic instability of the microtubule tips. We will reconstitute these transport systems in vitro using a minimal set of protein components and explore the self-assembly and behaviour of these systems with single molecule resolution using total internal reflection fluorescence (TIRF) and interferometric scattering (iSCAT) microscopy. Combining this experimental approach with mathematical modelling we aim to quantitatively explain some of the molecular mechanisms underlying microtubule-based intracellular transport.



## **Viral Nudix enzymes substrates and their role in infection**

Ing. Hana Macíčková Cahová, Ph.D.

ID 211768

Nudix enzymes are class of enzymes capable of cleaving nucleosidediphosphates linked to (any moiety). Their roles are not fully understood as they can cleave small molecules such as NAD and CoA as well as canonical RNA caps. Recent discovery of NAD and CoA as new RNA caps leads to reassessment of the RNA structure and Nudix enzymes roles. Some DNA viruses also code for their own Nudix enzymes. The task of this thesis is to define the real substrate of the viral Nudix enzymes and understand the role of viral Nudix enzymes in the infection.

## **Role, biogenesis and biodegradation of new RNA caps in prokaryotes**

Ing. Hana Macíčková Cahová, Ph.D.

ID 211769

Recent discovery of RNA caps such as NAD and CoA leads to reassessment of RNA structure in all types of cells. In our search for new RNA modifications, we have discovered new 5' caps. The task of the thesis is to reveal the role, biogenesis and biodegradation of these new caps. The second task is to develop technique that will allow for identification of particular capped RNA.

## **Development of neural crest cells in zebrafish *Danio rerio***

RNDr. Ondřej Machoň, CSc.

ID 212277

Buňky neurální lišty představují velmi zajímavou populaci kmenových buněk, které dočasně vznikají v nervové tkáni embrya, odkud se odštěpí a migrují do mnoha míst a tkíní v zárodku. Přes jejich neurální původ tyto buňky diferencují do mnoha odlišných buněčných typů: hlavové nervy, enterické nervové buňky, pigmentové buňky, hladké svaloviny, srdeční chlopně či hlavové chrupavky a kosti. Je proto velmi zajímavé sledovat diferenciační potenciál buněk neurální lišty během migrace do cílových tkání a studovat mechanismy genové regulace při jejich vývoji. V naší laboratoři jsme nedávno etablovali CRISPR/Cas9 systém na mutagenezi v zebříčkách *Danio rerio*. Například jsme připravili mutované rybí linie Meis1a, Meis1b, Meis2a a Meis2b, což jsou transkripční faktory, které jsou aktivní v buňkách neurální lišty a významně ovlivňují genovou expresi při jejich diferenciaci v myším embryu (Machon et al., 2015). Naše předběžné výsledky z pokusů s morfoliny v zebříčkách *Danio* ukazují na zásadní roli faktorů Meis také při vývoji kraniofaciálních struktur také u ryb. Student se zaměří na podrobnou analýzu morfologických změn u genetických mutant. Metodicky využije imunohistochemické analýzy, micro CT (počítačovou tomografii), in situ hybridizaci a CRISPR/Cas9 mutagenezi pro tvorbu nových linií *Danio*.

## **Impact of PPM1D/Wip1 phosphatase on chromatin organization in human cells**

MUDr. Libor Macůrek, Ph.D.

ID 212620

Genome instability caused by defects in DNA repair can contribute to various pathologies including cancer. Genome integrity is protected by coordinated action of molecular pathways that control the cell cycle and DNA repair. In addition to the signaling at the DNA break, DNA damage also affects organization of the chromatin at pan-nuclear level. This project aims to study changes in the chromatin organization caused by genotoxic stress.

Protein phosphatase PPM1D/Wip1 is localized at chromatin and inactivates cellular response to DNA damage. Here we will address the impact PPM1D/Wip1 phosphatase on chromatin organization in human cells. In particular we will compare distribution of the heterochromatin in control cells, cells where PPM1D/Wip1 was inactivated by CRISPR/Cas9 technology and in cells treated with a small molecule inhibitor of PPM1D/Wip1. Chromatin organization will be studied in normal conditions and after induction of DNA damage using various molecular and cell biology techniques. We expect that this project will contribute to better understanding of cellular responses to DNA damage.

We seek a motivated PhD student to join our young international team located in Prague, Czech Republic. We are especially interested in candidates with strong background in molecular/cell biology or biochemistry and with enthusiasm for basic science and experimental work.

## **Impact of mitochondrial DNA diversity on metabolic phenotype and innate immunity**

RNDr. Tomáš Mráček, Ph.D.

ID 211571

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.

Candidate's profile (requirements):

We are seeking for highly-motivated person with MSc. or equivalent degree in cell biology, biochemistry, immunology, physiology or similar field obtained before or during 2019. Candidate should be fluent in English and apart from the experimental "wet" work, she/he should be willing to work with laboratory animals.

## **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.

## Role of Cullin3 in embryonic development

Mgr. Jan Procházka, Ph.D.

ID 212157

Ubiquitination is a process of post-translational protein modification involved in most of the cellular processes. It is essential for development. Moreover, a key role of ubiquitin ligase is to target its modified substrates for proteasomal degradation [1]. Cullin-RING ubiquitin ligases (CRLs) represent the largest family of ubiquitin E3 ligases. The C-terminal domain of Cullins is highly conserved and covalently modified by the activator NEDD8 [2]. The N-terminal domain is variable among family members and Cullins use this region to interact with their specific adaptor proteins. For CRL3 it is bric-a-brac, tramtrack and broad complex (BTB) protein. BTB domain identified in these proteins, originally known as the Pox virus and Zinc finger (POZ) domain, often contains a second protein-protein interaction motif such as Kelch or Zinc finger motif [3]. So far, only approximately 50 out of 180 BTB proteins encoded in the human genome have been confirmed as substrate receptors in CRL3 complexes [4]. Distinct CRL3 complexes have been described to play a role in myogenesis [5], neurogenesis [6], chondrogenesis [7], and osteogenesis [8], however role of CRL3 complexes in embryonic development is largely unknown. Cul3 KO embryos exhibit early lethality by embryonic day E7.5 which is associated with disorganization of the early extraembryonic tissues differentiation, absence of amnion and the extraembryonic cavities [9]. The goal of this PhD project will be to evaluate a role of Cullin3 during embryonic development with focus on craniofacial development and identification of its developmentally regulated interaction partners. Despite the early lethality, the role of Cullin3 can be studied by using conditional tissue-specific and inducible Cre knockout lines. To study the role of Cul3 in development of cranio-facial area, we will use the Wnt1-Cre driver and conditionally inactivated Cul3 in neural crest and its derivatives, e.g. facial cartilage and bone, PNS glial cells or odontoblasts. Fgf8-CreER driver will be used to conditionally inactivate Cul3 and to study its role in the teeth and limb development. We will evaluate the genotype ratio during breeding and to detect any abnormal anatomy, not visible by simple observation, we will use high resolution micro-CT imaging. Acquired virtual 3D sections then can be compared with histological sections. Specific organ development will be analysed with deep stack confocal imaging with use of 2P confocal microscopy and spinning disk confocal live imaging. In order to reveal molecular pathway downstream from CRL3 complex in embryonic development we will use molecular biology and biochemistry methods to identify interacting partners of Cul3, by co-immunoprecipitation from developmentally relevant tissues followed by mass spectrometry and immunoblotting. From possible interacting partners we will focus on Btb family which has not been experimentally proved as Cul3 associated factors yet. We will use knowledge of embryo phenotyping procedures and proceed advanced embryo phenotyping for Btb1, Btb3, Btb6, Btb7, Btb8 and Btb9 KO lines produced by Prof. Sedlacek's at CCP. The direct interaction between Btbs and Cul3 will be tested on cell cultures by tagged proteins, the cell culture results will be always validated in developmental context. As main result of project the identification of role Cul3 ligases in morphogenetic processes during embryonic development shall shed more light to specific Cul3-substrate adaptor interactions in normal physiological processes and broaden understanding of Cul3 role under pathological conditions. This work will be covered by Assoc. Prof. Radislav Sedlacek. His Laboratory at the Institute of Molecular Genetics has all the necessary funds, equipment and conditions for successful completion of the planned PhD project.

## **Deciphering CRL4 complex interacting pathways in development and tissue homeiostasis**

Mgr. Jan Procházka, Ph.D.

ID 212158

Cullin-4A (Cul4a) is a member of the cullin-RING ubiquitin ligases (CRLs), the largest E3 ligase family present in eukaryotic organisms, playing an important role in event of gene expression, embryonic development, signalling, DNA damage response or cell cycle. Cul4a itself serves as a scaffold for assembling functional E3 complexes in the ubiquitination process (Pickart, 2001). Cullins are connecting substrates via the receptor joined to the cullin complex by a linker protein (Zheng et al., 2002), such as DDB1, a DNA damage-binding protein 1, interacting with DDB1 and Cul4a-associated factors (DCAFs) (Nag et al., 2001). An example of such DCAF protein is Cereblon (CRBN), a protein important for the limb (Ito et al., 2010) and cerebral (Xin et al., 2007) development. The C-terminal domain of Cul4a interacts with other proteins, for instance a small RING finger protein ROC1 (ring of cullins) (Ohta et al., 1999), which is recruiting the E2 enzyme of the ubiquitin pathway and thus activating it, and NEDD8, a ubiquitin-like protein activating Cul4a (Osaka et al., 1998). Cul4a was previously studied and described as an over-amplified gene in human breast cancer (Chen et al., 1998) and more recently also in ovarian cancer, lung cancer as well as other solid tumours (Beroukhi et al., 2010). But apart from Cul4a role in the limb development (Ido et al., 2010), however interestingly its part in the development and physiology remains unknown. The goal of this PhD project is to identify the interaction network during development and physiological processes directly in tissues and organs, specifically with focus on epithelial organ development and regeneration. The classical biochemical methods do not always provide sufficient accuracy in specific tissue samples. Project will take advantage of in vivo tagging system – BioID technique. This method is using a promiscuous biotin ligase, fused to a protein of interest, to biotinylate endogenous proteins based on proximity. This biotinylation enables selective isolation of these endogenous proteins, prospective interactors with the protein of interest and their identification with biotin-affinity capture (Roux et al., 2012). However, such approach can't help to identify development and tissues physiology regulating networks since is predominatly used only in cell lines. In order to overcome this obstacle the major aim of project is to introduced in vivo tagging system into experimental mouse model. A targeting of embryonic stem cells will be used in this project to establish transgenic mouse models for studying Cul4a complex in embryonic and adult tissue, such as Cul4a-BioID and Crbn-BioID knock-in models using CRISPR/Cas9 system. Special focus will be devoted to pharyngeal arches and limb buds to evaluate the Cul4a interaction partners in cul4a development regulation network. Other tissue types will be studied upon the determination of Cul4a expression in different tissue types with LacZ staining, whole-mount in situ hybridization or immunofluorescence. Additional techniques will be used to validate the results from BioID analysis, like Tandem Affinity Purification combined with Mass Spectrometry, Yeast-Two-Hybrid assays, Fluorescence Resonance Energy Transfer assay or Co-Immunoprecipitation. Once the interactions within the complex are defined, other experiments can be done on primary embryonic tissue cultures or organoid cultures, such as knock-down of gene expression by RNAi or other genome editing tools, to determine the interaction hierarchy. Clarification of protein interactions within this complex can contribute greatly to resolve the regulatory functions of Cul4a protein. Laboratory of Assoc. Prof. Radislav Sedlacek at the Insitute of Molecular Genetics, where this work will be conducted, has all the necessary funds and equipment for successful completion of the proposed doctoral project.



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

doc. RNDr. Daniel Rösel, Ph.D.ID 212239

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.

## **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.

## **The role of new candidate genes in glucose homeostasis and insulin sensitivity**

doc. RNDr. Radislav Sedláček, Ph.D.

ID 212244

Among several other genes, C4orf22 and CNBD1 were recently found to show a link to dysfunctional glucose homeostasis and impaired insulin sensitivity both in knockout mutant mice as well as in genome wide association studies in humans (Rozman et al. 2018). The exact physiological roles of these genes and their involvement in the development of metabolic disorders on the genetic and molecular level are unknown so far. The project will focus on the in-depth characterization of one or two candidate genes both on the physiological and the molecular level. The candidate will make use of different mouse models with the aim to characterize new targets for the diagnosis and possibly treatment of diabetes.

## **Postnatal plasticity of the cardiac conduction system**

prof. MUDr. David Sedmera, DSc.

ID 212200

The project involves studies of postnatal plasticity of conduction system in mouse and rat models.

The candidate will be enrolled in Developmental and Cell Biology study sections. The lab is supported by Charles University PROGRES (Q38) and several Czech Science Foundation grants.

Techniques used include optical mapping, immunohistochemistry, and confocal microscopy with 3D reconstructions. There is also a possibility to participate on Anatomy teaching (in Czech or English) to enhance your portfolio of skills.

## **Assembly of the spliceosome**

doc. Mgr. David Staněk, Ph.D.

ID 211737

RNA splicing, a key step in eukaryotic gene expression, is catalyzed by one of the most complex and dynamic particles in the cells – the spliceosome. The spliceosome consists of several building blocks, each of them contains non-coding RNAs and dozens of proteins. These ribonucleoprotein building blocks do not assemble spontaneously but require a number of auxiliary proteins that promote their correct formation and control the quality of the assembly process. The main objective of this PhD project is to investigate, utilizing a wide-range of life-cell imaging microscopy techniques in combination with molecular biology and biochemistry approaches, how are spliceosomal components correctly assembled in the dynamic and complex cell environment.

## **Epigenetics and genetics at the intersection of environment and human disease**

prof. MUDr. Tomáš Stopka, Ph.D., konzultant Ing. Jiří Zavadil, Ph.D.

ID 211790

The maintenance of human health results from dynamic interactions of extrinsic and intrinsic forces involving the environmental stimuli, the genetic background as well as its epigenetic realization into different cellular products from which phenotypic manifestations arise. Clonal disorders such as solid tumors and hematological malignancies are results of alteration of these homeostatic interactions. In our collaborative work, we seek to understand causative as well as other pathogenic molecular factors operational in cancer development, and to provide rationale for disease prevention as well as foundations for overriding therapeutic resistance.

The aims of the proposed project are:

- 1) to delineate genetic and functional imprints in response to carcinogens;
- 2) to identify modifiable epigenetic variables that can help preventing the impact of cancer-causing processes.

Innovative transgenic murine and cultured cell systems, modern cellular and molecular methods including genome-scale next generation sequencing, and the development and testing of novel therapeutic agents are employed in our research program. We seek scientifically curious and talented PhD students to join the Czech (BIOCEV) as well as international (IARC) modern laboratory environments to develop conceptually novel projects in the above research areas.

## **Cellular and organismal roles of the rhomboid intramembrane protease RHBDL4**

Ing. Kvido Stříšovský, Ph.D.

ID 213364

Intramembránové proteasy z rodiny rhomboidů regulují u octomilky signalizaci přes receptor pro epidermální růstový faktor, ale jejich hlavní funkce u myši a člověka jsou pravděpodobně jiné a zůstávají nedostatečně pochopené. Proteasa z rodiny rhomboidů RHBDL4, lokalizovaná v endoplasmatickém retikulu (ER), se pravděpodobně uplatňuje při degradaci proteinů spojené s endoplasmatickým retikulem (ERAD), při sekreci exosomů a apoptose a je spojována s rakovinou tlustého střeva. Mechanismy zodpovědné za tyto funkce nebo jejich fyziologický význam jsou však kontroverzní nebo neznámé. Navrhujeme použít proteomickou identifikaci substrátů a proximitního proteomu RHBDL4 a funkční analýzu sekreční dráhy v buněčných kulturách k identifikaci molekulárních procesů, kterých se RHBDL4 účastní. Ve spojení s detailní charakterizací imunitního fenotypu RHBDL4 deficientní myši a s ex-vivo imunologickou analýzou primárních buněk nám to umožní odhalit fyziologickou funkci RHBDL4 a nové regulační mechanismy v sekreční dráze relevantní pro funkci imunitního systému a roli RHBDL4 v rakovině tlustého střeva.

## **Molecular and cellular basis of the catarrhal phase and transmission of pertussis**

Prof. 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 donedávna 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 mutagenese patogena *B. pertussis* a pomocí genetické manipulace myši teď 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 transmissi 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 projektu 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.



## **Localization of mRNAs and lncRNAs during development**

Mgr. Radek Šindelka, Ph.D.

ID 211748

The study of the localization of RNA molecules has become an intriguing area of interest for the fields of Biochemistry, Biology and Medicine. It holds the potential to provide better comprehension on aspects of cancer formation, stem cell biology and organism development. Our laboratory is currently one of the leading groups studying asymmetrical localization of RNAs and proteins during the early developmental stages. We strive to keep up with the continual advancement in technologies, and are currently utilizing some of the state-of-the-art methods such as reverse transcription PCR (RT-qPCR) and RNA-sequencing (RNA-seq) at both the single cell and subcellular levels. Our researchers are frequently invited to present their research at renowned international meetings. We have established and maintained several collaborations involving experts in the fields of proteomics and bioinformatics (mainly international). Additionally, we keep and maintain our own amphibian model in the laboratory and collaborate with several other laboratories that utilize fish and mammalian models. Even though the project is challenging, there are many opportunities for motivated and research oriented students to find their own specialization and develop their scientific carriers. We offer well equipped laboratory and international team of young scientists.  
([www.labgenexp.eu](http://www.labgenexp.eu))

## **Chromosome dynamics and integrity in mammalian oocytes and early embryos**

doc. RNDr. Petr Šolc, Ph.D.

ID 212031

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 the developmental defects (e.g. Down syndrome) or infertility. Spindle apparatus is essential molecular machine ensuring normal chromosome segregation. In somatic mitotic cells bipolar spindle formation is supported by two centriole containing centrosomes. However, in oocytes multiple acentriolar microtubule organizing centers (MTOCs) facilitate spindle formation and finally MTOCs are 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 with each other 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 zygote (fertilized oocyte) to blastocyst spindle gradually shifts from meiotic to mitotic form and 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 can 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 work during meiotic maturation on already condensed chromosomes what is in strong contrast to somatic cells where DNA repair is suppressed in mitosis. Now, we would like to uncover how the absence of DNA damage checkpoints not only in oocyte but also in early embryos affects genome integrity in the beginning of the new life and why checkpoints are not working 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 in the right moment we would like to move to human oocytes to see how does it work?

More info at <https://owncloud.cesnet.cz/index.php/s/1c8wbcO1dZAOr4t>

## **Reprogramming of translation during oogenesis and embryogenesis**

Ing. Andrej Šušor, Ph.D.

ID 211775

A subset of maternal transcripts is stored in a dormant state in the oocyte, and the timely driven translation of specific mRNAs guides meiotic progression, the oocyte-embryo transition, and early embryo development. In the absence of transcription, the regulation of gene expression in oocytes is controlled almost exclusively at the level of transcriptome and proteome stabilization and at the level of protein synthesis. Gene expression and cell cycle control are tightly controlled process in every cell. Recently, studies shows that, tightly controlled translational program occurring during the cell cycle, thus understanding the molecular mechanism of translational control through cell cycle assume very important. We analysed activity of positive translation initiation, elongation factors, and their repressors during interphase and M-phase of meiosis or mitosis of germ cells and early embryos. We found that number of key players in the translational machinery change their actions based on the cell cycle stage. Here we found that after resumption of oocyte meiosis or entering to the first and second mitosis of early mouse embryos, the translational program significantly reprograms to specifically regulate the expression of the subset of transcripts which are necessary for mammalian oogenesis and early embryo development.

## **Targeting hematological malignancies using fragment-based drug design**

Ing. Václav Veverka, Ph.D.

(i fyzikální chemie)

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.

## **Structural relationship between subunits of chromatin remodeling complexes**

Ing. Václav Veverka, Ph.D.

ID 213198

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.

## **Transient nuclear protein-protein interaction networks**

Ing. Václav Veverka, Ph.D.

(i fyzikální chemie)

ID 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.

## **Analyzing mechanisms of Huntington disease pathogenesis using human cell lines and transgenic animal models.**

Mgr. Petr Vodička, Ph.D.

ID. 212170

Huntington disease (HD) is a fatal hereditary neurodegeneration, caused by an expansion of CAG tract in IT15 gene to over 36 repeats. This repeat codes for extended stretch of glutamines in N-terminal part of huntingtin protein (HTT), changing its biochemical properties and causing aggregation and toxicity especially to striatal neurons. Exact mechanisms leading to selective vulnerability of neural cells to mutant huntingtin (mHTT) toxicity as well as normal cellular function of HTT are still largely unknown. The proposed project aims to analyze the changes in composition of in vitro differentiated neural cell proteome in the presence of mHTT, with special focus on cell surface and secreted proteins, which contribute to cell-to-cell communication. This will bring new knowledge about developmental and pathologic changes caused by the presence of mHTT and will contribute to better understanding of cell autonomous and intercellular mechanisms of HD pathogenesis. Selected phenotypes will be also studied using animal models transgenic for mHTT (mouse, minipig).

Methods: Tissue culture (iPS cells, neural stem cells, neurons), molecular biology (molecular cloning, transfection, qPCR), biochemical methods (western blot, ELISA, Luminex xMAP), mass spectrometry proteomics (LC-MS), confocal microscopy.

## **A role of Stress granules in the modulation of cell signalling 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)



## **The role of the ERK signaling pathway 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.

## **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 live-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.

## **Mechanisms of of chronic myelogenous leukemia cell resistance to tyrosinkinase inhibitor therapy.**

doc. MUDr. Daniel Vyoral, CSc.

ID 211740

Zásahová místa buněk chronické myelodní leukemie (CML) rezistentních k tyrozinkinázovým inhibitorům (TKI).

Chronická myelodní leukémie je nádorové onemocnění krvetvorby unikátní v tom smyslu, že příčina zvratu nádorových buněk tkví ve vzniku jediného nového fúzního proteinu - Bcr/Abl v těchto buňkách v důsledku chromozomální přestavby jejich genomu. Tento onkoprotein pak ovlivňuje množství buněčných procesů vedoucích k nádorovému zvratu těchto buněk. V současnosti je většina nemocných s touto chorobou úspěšně léčena selektivními inhibitory kinázové aktivity proteinu Bcr/Abl (TKI), avšak u části nemocných dochází po určité době léčby ke vzniku rezistence.

Naším cílem je objasnit pomocí proteomických technik mechanismy rezistence nádorových buněk CML na TKI a studovat molekulární mechanismy poskytující selektivní výhody leukemickým kmenovým buňkám.

Využíváme modelů nádorových buněk s vyvinutou rezistencí proti TKI. Unikátnost linií spočívá v tom, že rezistence těchto buněk k TKI není způsobena mutací v genu kódujícím protein Bcr/Abl. Tento buněčný model tedy umožňuje identifikovat na Bcr/Abl nezávislé mechanismy rezistence.

U tohoto modelu leukemických buněk provádíme "label free" proteomickou analýzu. Tyto pokusy již poskytly řadu zajímavých kandidátů – klíčových bílkovin a proteinových komplexů, které jsou nutné pro vznik rezistence. Tyto mohou představovat cenná terapeutická zásahová místa.

Zabýváme se dále biologickou funkcí buňkami uvolňovanými váčků – exosomů v normální a nádorové krvetvorbě a biologií normálních a nádorových krvetvorných kmenových buněk.