OR Vývojové a buněčné biologie

Vypsané doktorské práce pro akademický rok 2023/2024

Targeting mitochondrial fatty acid oxidation and adipose tissue browning to prevent obesity

RNDr. Lukáš Alán, Ph.D.

ID 258460

Almost 60% of the European Union population is overweight with around 25% obese people. A higher BMI is a major risk factor for metabolic disorders such as type 2 diabetes mellitus, hypertension, and cardiovascular diseases. There are several possibilities for obesity treatment, but they mostly require an active approach from the affected person (dietary changes, physical activity, recovery after surgery, weight loss medication). Among other therapies, hormonal treatment or browning of white fat cells represent promising approaches for patients. The browning is accompanied by mitochondrial biogenesis and increased capacity for fatty acid oxidation. In the proposed project, we plan to study the role of mitochondrial proteins in the browning of subcutaneous adipose tissue and uncover their potential for treating obesity and metabolic syndrome. The study will be performed in various experimental models e.g., cellular KO´s, primary cell lines and mouse KO models. For example, we will use models with genetically eliminated Vwa8 protein - Hek 293 Vwa8 KO cells show respiration-dependent substrate preference towards mitochondrial fatty acid oxidation and Vwa8 KO mice are characterized by the browning of subcutaneous adipose tissue, increase in fatty acid oxidation and improved insulin sensitivity. New data characterizing mitochondrial fatty acid oxidation and browning of subcutaneous adipose tissue may contribute to obesity treatment.

Metabolic reprogramming of cancer cells upon changes in style of migration

ID 257600

prof. RNDr. Jan Brábek, Ph.D.

The ability of cells to invade and metastasize belongs among the hallmarks of cancer, as defined by Weinberg and Hanahan. During dissemination from a primary tumor, cancer cells invade the ECM most commonly in clusters or sheets, what is referred to as collective migration, which requires proteolytic degradation at the invasive front and cell contractility in the following cells. Alternatively, single cancer cells can detach and invade using protease-dependent mesenchymal migration or protease-independent amoeboid migration, or combination of both. Further, many cancer cells can actively switch between these invasion modes in response to changes in the surrounding environment and/or to escape therapy. Within the primary tumor site, metabolic differences divide cells into distinct subpopulations that have unique capabilities enabling them to proceed through the metastatic cascade. Additionally, because cells are reprogrammed at different stages of metastasis to rely more on glycolysis or oxidative phosphorylation, it is crucial to understand which pathway is dominant at each stage. The aim of this project is to elucidate the link between cancer metabolism and different modes of migration in both 2D and 3D conditions, since it has been demonstrated that metabolism in 3D spheroids differs significantly from what is measured in 2D cultures, both in terms of glycolytic and oxidative phosphorylation metrics. To achieve this goal, we intent to analyze cancer cell migration and invasivity after inhibition of OXPHOS and/or glycolysis in 2D and 3D in cancer cells exhibiting different modes of migration. The project also aims to examine the metabolic reprogramming of cancer cells during the mesenchymal-to-amoeboid transition and vice versa.

Environmental regulation of membrane-associated structures in human blood cells

Mgr. Marek Cebecauer, Ph.D.

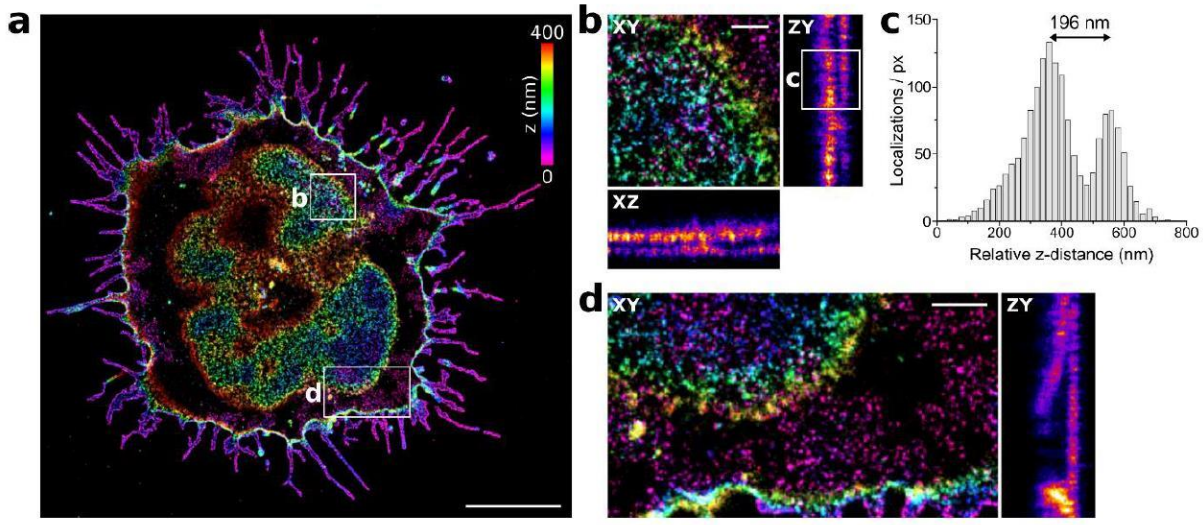
ID 257986

Cells employ diverse armoury to protect organisms from injuries, infections and other harmful conditions. A majority of these structures are associated with the plasma membrane and thus exposed to the environment. The best described protective structures in humans are phagocytic sites and the synapse formed between lymphocytes and target cells. Even though less well understood, neutrophil extracellular traps (NETs) belong to the most common tools to prevent microbial infections 1. Similarly, small protrusions on the surface of majority of cells – microvilli – are key structures regulating protective responses to pathological events.

In this project, student will use two types of experimental approaches to determine how environmental factors affect NETs and microvilli of human neutrophils and T cells. First, standard cell-biological and biochemical techniques (standard microscopy, flow cytometry, immunoblotting – available in the Co-Supervisor's lab) will be used to find conditions modifying function of these structures. Second, high-end microscopy approaches (available in Supervisor's lab 2) will be used to learn about nanoscopic changes, which led to the altered function. Together, these studies should help to understand how environmental factors such as oxidative stress or temperature influence nanoscopic structures essential for a complex response of immune cells to the adverse conditions.

1. Papayannopoulos V. Neutrophil extracellular traps in immunity and disease. Nat Rev Immunol 18: 134 (2019) DOI: 10.1038/nri.2017.105

2. Franke C; Chum T; Kvicalova Z; Glatzova D; Gentsch GJ; Rodriguez A; Helmerich DA.; Herdly L; Mavila H; Frank O; Brdicka T; van de Linde S and Cebecauer M. Approach to map nanotopography of cell surface receptors. Comms Biol. 5: 218 (2022); DOI: 10.1038/s42003-022-03152-y



Morphology of mitochondria and their membrane contact sites in pancreatic β-cells

Ing. Andrea Dlasková, Ph.D.

ID 256241

Mitochondrial metabolism is crucial for pancreatic ß-cells physiology. According to the dogmatic scheme of insulin secretion, increased ATP production by mitochondria leads to the closure of ATP-sensitive K + channels, membrane depolarization and opening of voltage-gated Ca 2+ channels. Ca 2+ influx into the cell subsequently leads to the secretion of insulin granules. However, in addition to ATP production, mitochondria have many other functions and also function as important signaling domains. Mitochondrial activity and mitochondrial structure are very closely linked, but the mechanisms are not yet known. The aim of this dissertation will be to investigate how the ultrastructure and morphology of mitochondria influence ATP production, insulin secretion, viability and other factors crucial for pancreatic ß-cell physiology. We will use the regulation of gene expression (siRNA, overexpression, CRISPR/Cas9 edited cell lines) of proteins that are key to cristae formation (ATP synthase oligomerization subunits, inhibitory factor IF1, OPA1 protein). In parallel with the study of mitochondrial ultrastructure, we will analyze the formation of membrane contacts between mitochondria and the ER/nuclear envelope, which also fundamentally regulate pancreatic ß-cell physiology.

Screening for proteins involved G4-structure metabolism and their role in prevention of genotoxic stress

RNDr. Jana Dobrovolná, Ph.D.

ID 257819

DNA replication is an essential and one of the most complex processes in the cell that ensures faithful, complete and timely duplication of genome. Not only exogenous DNA damage but also intrinsic DNA structures including G-quadruplexes (G4) and R-loops, their stabilization or unscheduled formation represent major replication obstacles with possible detrimental effects on genome integrity. Not surprisingly, those processes are pharmacologically targeted in anticancer therapy, despite the fact that only little is known about the underlying molecular mechanisms. It becomes apparent that maintenance of processive DNA replication requires sophisticated protein networks beyond the core replisome. Whether there is a direct crosstalk between G4 and R-loops, what proteins are involved in their homeostasis and what are the factors diversifying between their beneficial and pathological roles is not well understood. The goals of our research are to identify proteins associated with G4 and R-loop structures and understand their roles in G4/R-loop formation and resolution as well as relationship to replication fork progression and associated repair. PhD student will be involved in preparation of tools for study of G4 structures (establishment of various cell lines) and in identification of proteins involved in metabolism of these structures by mass spectrometry-based proteomics approaches, including APEX-based proximity labeling and chromatin affinity precipitation methods, and by functional siRNA screen. Proteins identified in these screens will be selected for further validation and characterization based on their relevance to G4/R-loop and replication fork metabolism, and role in maintenance of genome integrity.

Chromosome dynamics and integrity in mammalian oocytes and early embryos

RNDr. David Drutovič, Ph.D.

ID 257906

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.

Development of scaffolds for a regeneration of bones and osteochondral defects

Mgr. Eva Filová, Ph.D

ID 254213

The aim is to develop biodegradable scaffolds which are able to release bioactive substances supporting formation of new bone or cartilage tissues (e.g. growth factors, peptides, exosomes from stem cells or plant cells), chemicals supporting angiogeneis or drugs (e.g. antibiotics).

Foam scaffolds based on collagen, tricalcium phosphates (alpha-TCP, beta-TCP, hydroxyapatite (HA), ceramic scaffolds based on TCP and HA with different nanostructure or microstructure. Moreover, composites with nano/microparticles will be prepared for a drug delivery.

Scaffoolds will be characterizated with physico-chemical methods, SEM etc., seeded with cells - cell lines, stem cells, coculture with other cell types, and tested from cell adhesion, proliferation and differentiation. Selected scaffolds will be tested in vivo (on rats, rabbits, pigs) and evaluated histologically.

Study of amino acid indispensability throughout bacterial proteome

Mgr. Klára Hlouchová, Ph.D.

ID 257757

The aim of this project will be to compare the substitutability of selected amino acids within selected metabolic pathways and a complete bacterial proteome. The motivation to this project is (i) to evaluate the indispensability of Tryptophane – the evolutionary latest and metabolically most expensive addition to the amino acid alphabet, and (ii) to experimentally test how essential Trp has really become and whether cells could still function without it. The primary approach to this task will involve adaptive laboratory evolution. Auxotroph strains deficient in the biosynthesis of respective selected amino acids (such as Trp, Thr and Ile) will be exposed to decreasing amount of the amino acid supplemented in the growth media and the bacterium response to amino acid scarcities will be studied. The selection pressure will likely result in different types of response, ranging from translation errors to accumulated mutations throughout the genome. The genome sequence will be continuously monitored by whole genome sequencing over ~1,000 generations and the experiment will be performed with several parallel lineages. The sequencing analysis will be accompanied by proteome-wide amino acid analysis by liquid chromatography mass spectrometry to monitor translational errors. An alternative approach will include genome engineering targeting specific metabolic pathways within the E.coli proteome. The candidate for this project should have strong team-work and wet-lab skills (previous work with bacterial cultivation and evolution studies are welcome) and also basic bioinformatic competence.

In vitro evolution and engineering of XNA polymerases

prof. Ing. Michal Hocek, CSc., DSc.

ID 246675

XNA (xenobiotic nucleic acids) are modified artificial analogues of biopolymers orthogonal to natural nucleic acids (DNA or RNA). They mostly contain modified sugar part that makes them resistant to cleavage by nucleases and enables unique secondary folds. Natural DNA or RNA polymerases typically do not tolerate dual base- and sugar-modified nucleoside triphosphates (XTPs) as substrates which seriously limits the application of the XNA methodology. To this end, we propose a project that aims at the in vitro evolution of engineered XNA polymerases to enable efficient enzymatic synthesis of base-modified XNAs from modified XTPs bearing a portfolio of useful modifications. We will use best performing exonuclease-deficient Thermococcus gorgonarius (Tgo) polymerase variants as a platform for mutant library generation using transposon-directed mutagenesis and targeted mutagenesis. Having the library in hands, we will adopt established methods for directed evolution, e.g. phage display or emulsion-based selections. The ultimate and groundbreaking goal would be to develop a polymerase capable of robust synthesis of longer sequences of dual base- and sugar-modified XNA using full set of all-four-base-modified XTP building blocks. However, even a polymerase capable of incorporation of several base-modified nucleotides would be very desirable.

Exploring the molecular mechanisms involved in the resolution of transcription-induced replication stress

RNDr. Pavel Janščák, Ph.D.

ID 257823

Rationale: Transcription-replication conflicts (TRCs) associated with the formation of co-transcription RNA:DNA hybrids, termed R-loops, represent a major source of DNA replication stress. We identified a molecular pathway mediating replication restart at TRC sites to prevent aberrant chromosome segregation due to under-replicated DNA, which can lead to chromosomal rearrangements. Our data suggested that this pathway involves fork cleavage-religation cycles catalyzed by MUS81/EME1 endonuclease and the DNA ligase IV (LIG4)/XRCC4 complex, which eliminate the topological barrier in the DNA template generated by converging transcription and replication complexes, allowing resumption of fork progression. More recently, we identified the human DEAD-box helicase DDX17 as a factor that associates with R-loops and promotes the reactivation of R-loop-stalled forks via the MUS81-LIG4 pathway in a manner dependent on its helicase activity. In addition, our biochemical experiments revealed that DDX17 unwinds R-loops in vitro. Together, these data suggested that DDX17 might be involved in the elimination of R-loops at sites of R-loop-mediated TRCs to allow for the passage of the reactivated replication fork. Interestingly, our ongoing work has revealed that MUS81-initiated restart of R-loop-stalled forks requires an additional RNA/DNA helicase, termed Senataxin, which is encoded by the SETX gene whose mutations are associated with two progressive neurological disorders termed ataxia oculomotor apraxia 2 (AOA2) and amyotrophic lateral sclerosis 4 (ALS4). Moreover, we have found that SETX co-immunoprecipitates with DDX17 from human cell extracts, suggesting that these RNA/DNA helicases form a complex and may act in a coordinated fashion to eliminate R-loops at TRC sites, allowing fork progression. Here, we will explore the crosstalk between the DDX17 and SETX helicases in restarting R-loops stalled forks in human cells.

Research methodology and approach: By deletion mutagenesis, we will map the interaction site for SETX on DDX17 and then investigate whether mutational disruption of the DDX17/SETX complex impairs the reactivation of R-loop-stalled forks via the MUS81-LIG4 pathway. For this, we will establish a stable U2OS T-Rex cell line inducibly expressing an siRNA-resistant version of a DDX17 mutant that does not bind to SETX. As an alternative approach, the SETX-interaction domain of endogenous DDX17 will be inactivated by CRISPR-based genome editing in U2OS cells. By DNA fiber assay, we will investigate whether cells expressing this DDX17 mutant display transcription-dependent replication fork slowing and a failure to resume fork progression after treatment with R-loop-inducing drugs, phenotypes we identified upon DDX17 or SETX knockdown. In addition, by DNA:RNA hybrid immunoprecipitation (DRIP) followed by quantitative real-time PCR, we will test whether these cells accumulate R-loops at known R-loop-prone loci. To assess the epistatic relationship between DDX17 and SETX in suppressing R-loop-mediated replication stress, we will analyze the phenotypic consequences of DDX17 knockdown in U2OS SETX knockout cells. Moreover, by DRIP-Seq and INDUCE-Seq, we will determine whether DDX17 and SETX suppress R-loop accumulation and DNA breakage in the same genomic regions. As a parallel approach, the cooperation between purified DDX17 and SETX proteins in unwinding of model R-loop structures will be investigated.

Cytogenomika a editace genomu žab čeledi pipovitých

Ing. Martin Knytl, Ph.D.

ID 258035

Živočichové mívají konzervovaný fenotyp – pouze samčí nebo samičí pohlaví, ale geny, které jsou zodpovědné za to, jestli se embryo bude vyvíjet v samce nebo samici (spouštěče diferenciace pohlaví), se mezi jednotlivými druhy značně funkčně i strukturně liší. Tato genetická variabilita, která vede ke konzervovanému fenotypu se nazývá vývojový drift (z angl. developmental systems drift, DSD). Zatím jen málo spouštěčů diferenciace pohlaví bylo u obratlovců identifikováno, což brání našemu správnému chápání DSD diferenciace pohlaví. Cíle tohoto projektu jsou vyvinout a použít experimentální srovnávací cytogenetickou a genomickou sadu metod ke studiu evoluce pohlavních chromozómů a funkce genů souvisejících s genetickým určováním pohlavím u obojživelníků.

Molecular mechanisms of regulation of pancreatic β-cell function and viability in relation to type 2 diabetes pathogenesis

prof. RNDr. Jan Kovář, DrSc.

ID 255133

Dysfunction and apoptosis of pancreatic β-cells are among the key factors contributing in type 2 diabetes development. Many factors affect β-cells, especially low physical activity combined with unhealthy diet (e.g. chronically increased intake of saturated fatty acids). Some associated pathological states (e.g. sleep apnea leading to chronic hypoxia in pancreas) and environmental pollutants have negative effect on β-cells as well.

Exact molecular mechanisms by which the harmful factors affect β-cell function and viability are not elucidated yet. The aim of the project is to contribute to elucidation and understanding of these mechanisms.

Methods of cell and molecular biology (Western blot, FACS, confocal microscopy, siRNA, CRISPR, etc.) will be employed to study involvement of e.g. miRNAs, various signaling pathways, autophagy and alternative cell death pathways (e.g. ferroptosis, necroptosis). As experimental model, human and animal β-cell lines will be used. Alternatively, key results will be verified on isolated Langerhans islets.

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Effect of iron accumulation on the function of critical tissues

prof. RNDr. Jan Kovář, DrSc.

ID 255136

We have been studying molecular mechanisms of iron transport and metabolism in various types of mammalian cells for a long time.

The project is focused on the problematics of iron transport mechanism and cell damage/cell death in specific tissues as a result of iron accumulation. Cell lines as well as samples of patients with chronic iron overload diseases will be used. In the project, we will further study cellular functions and mechanisms of iron transport into cells in patients with diabetes mellitus or prediabetes, or in patients with heart failure. As a part of the project, it is also possible to monitor the effect of increased iron intake on the development of metabolic syndrome, as well as iron metabolism in tumor tissue.

We will use following experimental models: (1) cell lines of hepatocytes (HEP-G2, HepaRG), pancreatic beta cells (NES2Y, INS1E) and cardiomyocytes (H9c2) will be used (2) tissue samples from patients with impaired iron metabolism (alcoholic liver disease, hemochromatosis, anemia from iron deficiency, hepatitis, porphyria, etc.) and patients with impaired glucose metabolism and obesity. Methodologically, a wide range of cell and molecular biology approaches will be used.

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Pancreatic beta-cell and pollutants: the effect of pollutants on viability and function of pancreatic beta-cells

prof. RNDr. Jan Kovář, DrSc.

255138

Environmental pollution represents a significant threat to human health. Epidemiological studies suggest that, among others, pollution plays a role in the worldwide epidemy of diabetes mellitus. However, data is scarce, and pollutants' effects on pancreatic beta-cells remain largely unexplored.

The project focuses on exploring the effects of late (DDT, DDE, HCH) and present (TDCIPP, TPhP) pollutants on the viability and function of pancreatic beta-cells. Besides the production and synthesis of insulin, it will also explore the changes in the expression of proteins essential for beta-cell survival and functionality.

The project will use human, mouse, and rat beta-cell lines. Methods employed include western blot, ELISA, immunofluorescence, RT-PCR, and others.

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Molecular mechanisms of cancer cell resistance to chemotherapeutics

prof. RNDr. Jan Kovář, DrSc.

ID 255139

Resistance of cancer cells to chemotherapeutics represents a crucial problem of the therapy of cancer diseases. We are dealing with molecular mechanisms of resistance and mechanisms of its development. Together with that we are dealing with possibilities to overcome resistance of cancer cells by relevant newly constructed chemotherapeutics.

As experimental models, we use cancer cell lines, experimental tumors in mice and tumor samples from patients. Our interest is focused on cells of breast cancer and ovarian cancer. In the case of cell lines, we employ original cancer cell lines, which are sensitive to chemotherapeutics, and counterpart sublines with developed resistance to chemotherapeutics. We are interested in changes of expression of relevant genes in resistant cells, including changes concerning regulation of expression of these genes. In the case of one particular group of chemotherapeutics, i.e. taxanes, we are dealing with a construction of such derivatives of chemotherapeutics, n the basis of structural and functional studies, which are targeted to overcome resistance.

For our studies, we employ a wide range of methods of cell and molecular biology.

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Evolutionary history of vertebrate lateral plate mesoderm: an insight from the basal chordate amphioxus

RNDr. Zbyněk Kozmik, CSc.

ID 257836

Project will focus on evolution of cell types, ancestral chordate features and vertebrate-specific innovations, using comparative analysis between amphioxus and vertebrates. The methods used will include basic bioinformatics, gene expression studies (single cell RNA-seq, whole-mount in situ hybridization, and immunohistochemistry), analysis of gene knockouts established in the lab using CRISPR/Cas9 system, and reporter gene transgenesis.

Background:

Vertebrates have greatly elaborated the basic chordate body plan and evolved highly distinctive genomes that have been sculpted by two whole-genome duplications. The genome of invertebrate chordate amphioxus has not undergone whole-genome duplication and 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. 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. Moreover, recent publication on Amphioxus functional genomics and the origins of vertebrate gene regulation (Marletaz et al., Nature 564(7734):64-70) provides a huge genomic resource for future studies focused on gene regulatory mechanisms underlying evolution of vertebrate body plan.

Regulation of tau envelope by tau post-translational modifications

RNDr. Zdeněk Lánský, Ph.D.

ID 246454

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 role of tau post-translational modifications in envelope formation and function.

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.

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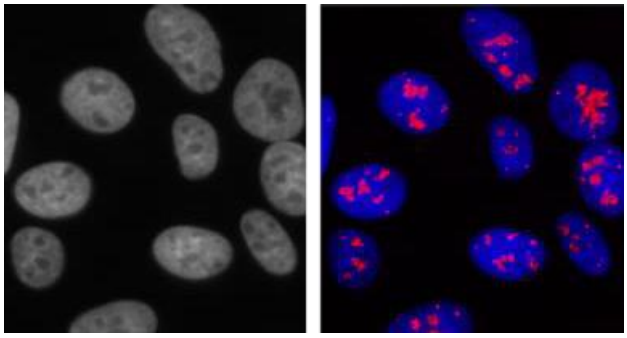
Candidate profile: We are looking for an enthusiastic PhD student motivated to work on cross-disciplinary projects. The candidate should hold a master's degree in (bio)chemistry, (bio)physics, molecular/cellular biology or an equivalent field.

Reverisble protein phosphorylation in control of nucleolar functions

MUDr. Libor Macůrek, Ph.D.

ID 257841

Nucleolus is a nuclear structure necessary for biogenesis of ribosomes. Nucleolus comprises of repeats of rDNA genes, RNA polymerase I complex and a large number of intrinsically disordered proteins that are prone to self-assembly by phase separation. Transcription of rDNA is a first step in ribosomal biogenesis and is precisely regulated to reflect the needs of each cell. Genotoxic stress temporarily inhibits rDNA transcription to prevent transcription-replication collisions. Broken rDNA genes are redistributed to nucleolar periphery to prevent harmful genomic rearrangements. These events are dynamically regulated by activities of protein kinases ATM and CK2 and the opposing protein phosphatases. This project will focus on novel role of protein phosphatase PPM1D/WIP1 in genesis of the nucleoli and rDNA repair. We will use gene editing technologies to knock-out/in genes in human cells, high-content microscopy to study localization and interactions of proteins in subcellular compartments, electron microscopy to study nucleolar morphology and in silico modeling to predict protein-protein interactions. The proposed project will improve our understanding of essential biological processes in the nucleoli.

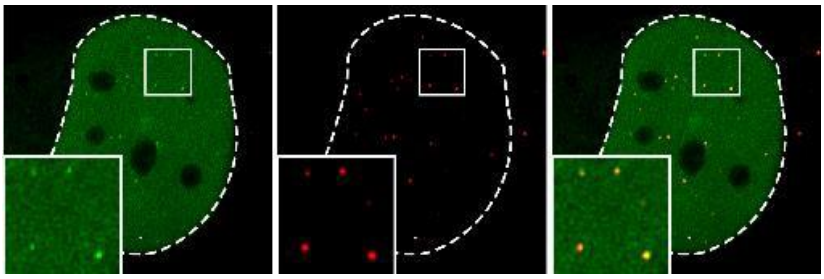


Phosphorylation of shelterin complex and its role in telomere maintenance

MUDr. Libor Macůrek, Ph.D.

ID 257842

Telomeres are nucleoprotein structures present at the ends of linear chromosomes that prevent activation of DNA damage response and protect cells from chromosomal fusions and genome instability. Shortening of telomeres causes senescence at cellular and promotes aging. Shelterin complex and its main component TRF2 binds to the repeats of telomeric DNA and promotes its folding into t-loop structure that allows protection of the single stranded DNA. Another component of shelterin complex recruits the telomerase that is needed for maintenance of the correct telomere length. Shelterin complex thus responds differently to various conditions and assembly of individual components needs to be dynamically regulated. We have recently identified new phosphorylation of TRF2 that affects its interaction with other shelterin subunits. This project will address how phosphorylation of the shelterin impacts the maintenance of the telomeres in human and mouse cells. We will use DNA combing assay to determine the speed of replication of telomeric DNA, high-content microscopy to detect marks of replication stress at telomeres and gene editing to evaluate contribution of mutant shelterin to the maintenece of telomere lenght. This project aims to address important biological function necessary for normal cell proliferation.



The role of SEL-5/AAK1 kinase in cell migration and cell growth

Mgr. Marie Macůrková, Ph.D.

ID 246671

Clathrin-mediated endocytosis is an important process involved in uptake of molecules into cells, signalling or membrane homeostasis. At the plasma membrane the clathrin pit assembly and cargo selection is assisted by adaptor proteins, among them the AP2 adaptor protein complex. In mammalian cells AP2 activity is regulated by AAK1 kinase phosphorylation. We utilized the nematode Caenorhabditis elegans to study the developmental role of AAK1 ortholog SEL-5 in this invertebrate model. SEL-5 activity is dispensable for general regulation of endocytosis in C. elegans, however loss of SEL-5 activity leads to a defect in migration of neuronal precursor cells and also to a defect in cell growth of a particular cell. Interestingly, defect in cell migration can also be observed in cultured mammalian cells after loss of AAK1 expression. AAK1 and SEL-5 thus seem to act in both similar and disparate mechanisms. The aim of this project is to dissect the mechanism of SEL-5 function in neuronal migration and in cell growth and compare the AAK1/SEL-5 mechanism of action between mammalian and C. elegans models. The project will combine advanced C. elegans genetic techniques including targeted CRISPR/Cas9 mutagenesis and in vivo protein tagging with biochemical and microscopic analyses in cultured mammalian cells.

Role of underlying mesechyme and neural network in the morhogenesis of epithelial placodes

RNDr. Ondřej Machoň, Ph.D.

ID 246289

Morphogenesis of some organs originating from the embryonic epithelium such as lens, hair, teeth or salivary glands is dependent on fine-tuned interactions between the surface ectoderm and the underlying mesenchyme, the neural tissue or the peripheral innervation. The peripheral innervation, which forms rather early in embryogenesis, may also serve to distribute signalling molecules influencing development of the neighboring epithelium. Molecular mechanisms of these interactions that determine spatiotemporal control of development of epithelial structures are still unclear. This project will analyze selected mouse embryo conditional mutants to unravel mechanisms of the formation of epitheliall structures and the role of neighboring tissues. The project will employ single-cell RNA-seq analysis, spatial transcriptomics, in situ RNA hybridization and immunohistochemistry. Advance microscopy techniques such as light-sheet or spinning disc confocal microscopy will also be included.

Prime edititing of the correction of mutation in the abca4 gene in the transgenic minipigs

prof. MVDr. Jan Motlík, DrSc.

ID 258251

The Center PIGMOD at IAPG CAS in Libechov, in a close coopreration with Professor Knut Stieger at the University of Gessen, Germany , created the unique model of the serious eye disease caused by mutation in ABCA4 gene – Stargardt disease.

The breeding strategy of these transgenic minipigs prepared a unique cohort of minipigs with the well characterized mutation. To correct this pathogenic mutation, it will be essential to identify an optimal prime-editing guide RNA paired with the corresponding target sequences.

The first part of this experimental effort will be done in vitro at the cultured porcine retina pigmented epithelial cells. After in in vitro experiments with cells transduced with the AAV vectors, the subsequent in vivo experiment will be realized in cooperation with vitroretinal surgeons front the Ophthalmologic Clinic of the University Hospital Kralovske Vinohrady in Prague. The optimalized prime editing sequences included in AAV vectors (Anc80) will be deposited in the subretinal space of WT and transgenic minipigs.

PhD study will be realized in a close cooperation with Dr. Tobias Wimmer , Department of Ophthalmology at the University of Giessen (see Nucleic Acid Ther 2023 Mar 1. doi: 10.1089/nat.2022.0037). It was prepared the bilateral grant application in WEAVE Program of GACR with a hope that this application will be supportive for this PhD study.

Applicants with a good training in molecular genetic methods as well as a full interest in biomedical research are welcome.

Molecular mechanisms of pathogenicity in ATP synthase disorders

RNDr. Tomáš Mráček, Ph.D.

ID 246649

Mutations in mitochondrial FoF1 ATP synthase responsible for 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.

References:

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.

Role of metaboplic switch in liver regeneration

prof. Ing. Jiří Neužil, CSc.

ID 248135

Liver is a highly intriguing organ due to its capacity to regenerate after partial resection (partial hepatectomy, PHx). In this PhD project, the student will study altered metabolic pathways following PHx, which is linked very fast proliferation of liver during its regeneration. Assumtions as well as preliminary data point to a switch from salvage pathways of pyrimidine and purine synthesis to de novo pathways, critically involving the mitochondrial function. For increased biomass needs during liver regeneraiton, there is need for fast synthesis of amino acids. All these metabolites use glutamin as the substrate. This implies that its synthesis will be powered by diversion of the uea cycle to aminatino of glutamate. This project will utilise advanced methods of biochemistry and cell biology, as well as stable isotope labelling followed by metabolomics, exclusively using mouse models of PHx.

Molecular basis and role of protein-protein interactions in the regulation of Apoptosis Signal-regulating Kinase 1 (ASK1)

prof. RNDr. Tomáš Obšil, Ph.D.

ID 257810

Apoptosis signal-regulating kinase 1 (ASK1) is a MAP kinase kinase kinase (MAP3K) that controls various responses to oxidative and endoplasmic reticulum stress and calcium influx via the p38 and JNK signaling pathways. Deregulation of ASK1 activity is involved in the development of many diseases, including neurological disorders, amyotrophic lateral sclerosis, cardiovascular disease, diabetes and cancer. The catalytic activity of ASK1 is regulated through a complex mechanism that involves interaction with a variety of different binding partners including thioredoxin (TRX), tumor necrosis factor receptor–associated factors (TRAF) and 14-3-3 proteins. Despite many years of intensive research, the molecular mechanism of ASK1 regulation remains unclear.

The aim of this project in the field of structural biology isto elucidate the molecular basis of ASK1 regulation and to understand the role of ASK1 binding partners in its regulation. For this purpose, the structure of ASK1 complexes with its binding partners TRX1, TRAF2 and 14-3-3 will be studied using integrative structural biology methods, in particular cryo-EM, SAXS, H/D exchange coupled to MS and chemical crosslinking coupled to MS. Together with the structure, the impact of these protein-protein interactions on the kinase activity of ASK1 will also be studied. All proteins will be prepared recombinantly using a bacterial expression system. Biophysical and structural experiments will be carried out in the laboratories of supervisor and co-supervisor (expression and purification of recombinant proteins, kinase activity measurements, DSF, SV AUC), at the core facilities of Biocev (HDX-MS, XL-MS) and Ceitec (cryo-EM), SAXS measurement will be performed at the DESY sychrotron in Hamburg, Germany. The project is funded by grants from the research group.

Regulatory mechanisms of Nedd4-like E3 ubiquitin ligases and the role of protein-protein interactions in their regulation

RNDr. Veronika Obšilová, Ph.D.

ID 257809

Nedd4-2 is a HECT E3 ligase that plays a critical role in pathophysiology by regulating multiple substrates, including the epithelial sodium channel (ENaC). Pathological consequences of Nedd4-2 dysregulation include respiratory distress, hypertension, electrolyte imbalance, and kidney disease. In addition to the not yet fully elucidated Ca2+ -dependent regulation of Nedd4-2, Nedd4-2 is regulated via phosphorylation and binding to various binding partners including scaffolding proteins 14-3-3. 14-3-3 proteins have the ability of binding the functionally different signal proteins, including kinases, phosphatases and transmembrane receptors by changing their function. The aim of this project is to elucidate the regulatory mechanism human (Smurf1, Nedd4L), yeast (Rsp5) HECT E3 ubiquitin ligases and to understand the role protein-protein interactions in their regulation. For this purpose, the structure of Nedd4L, Smurf1, Rsp5 complexes with different binding partners (ClC-5, 14-3-3 proteins, arrestins) will be studied using integrative structural biology methods, in particular cryo-EM, SAXS, H/D exchange coupled to MS and chemical crosslinking coupled to MS. The structure of these complexes will be solved by combining the results of experimental methods and methods of structural bioinformatics (if needed). The activity of E3 ligases will be measured using liposome-binding and ubiquitination assays. Combined, these approaches will allow us to elucidate regulatory mechanisms of Nedd4-like E3 ubiquitin ligases. This is a project in the field of structural biology and biophysical chemistry of proteins and is funded by grants from the research group. Cooperation with the Faculty of Science, Charles University in Prague.

The role of SURF1 protein in biogenesis of mammalian cytochrome c oxidase

Mgr. Petr Pecina, Ph.D.

ID 257886

Cytochrome c oxidase (COX) is a key enzyme of mitochondrial energetics providing ATP in mammalian cells. COX biogenesis integrates sequential assembly of 14 subunits, encoded by nuclear and mitochondrial genes. It depends on numerous ancillary factors, including SURF1 implicated in insertion of heme a into catalytic subunit COX1. However, its precise role remains unclear, although SURF1 dysfunction causes most frequent COX deficiency - fatal Leigh syndrome. Proposed project will follow up on our studies characterizing COX functional alterations in patients' fibroblasts harbouring SURF1 mutations, and on key preliminary discovery of incorrect cofactor (heme b) incorporation into COX in mouse SURF1 knock-out. We hypothesize that SURF1 represents an adaptor protein between heme a synthesis enzyme and COX1, that cooperates with putative homologues of assembly factors PET117 and Coa2 described in yeast, thus securing insertion of proper cofactor type. New data characterizing COX biogenesis may contribute to therapy of SURF1-associated neurodegeneration disease.

The experimental scope of the project would include biochemical analyses of COX hemylation (COX complex purification, HPLC analysis of hemes, native electrophoreses) in cellular and animal models (SURF1 KO), and characterization of the role of novel COX assembly factors (generation of cellular models using CRISPR-Cas9, proteomics, functional analyses of mitochondrial respiration).

Adaptace buněčného metabolismu vyvolané poruchami v oxidační fosforylaci

RNDr. Alena Pecinová, Ph.D.

ID 257620

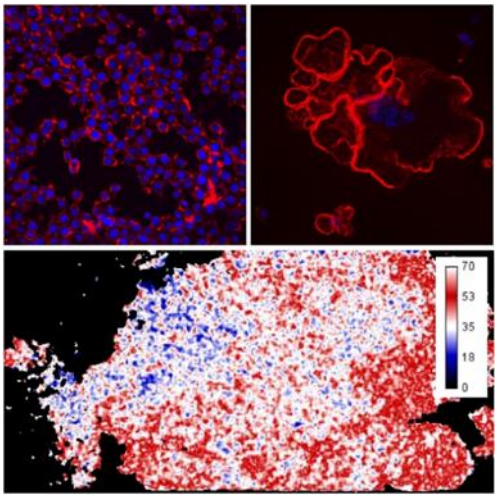
Mitochondrie produkují většinu buněčného ATP systémem oxidační fosforylace (OXPHOS) a poskytují meziprodukty pro biosyntetické dráhy. Poruchy OXPHOS systému vedou u lidí k vážným mitochondriálním onemocněním, které jsou způsobené narušením metabolické rovnováhy a postihují zejména tkáně s vysokými energetickými požadavky. Jelikož jsou OXPHOS enzymy kódovány jaderným i mitochondriálním genomem, je genová terapie velmi obtížná a výzkum se proto zaměřil na léčbu, která metabolický stres zmírňuje. Aby postižené buňky udržely metabolickou rovnováhu, jsou nuceny adaptovat svůj metabolismus. Spektrum metabolických adaptací je ale velmi široké a záleží nejen na druhu poruchy, ale i na její závažnosti.

V tomto projektu budeme zkoumat unikátní savčí modely s primárními poruchami OXPHOS systému. Pomocí moderních metabolomických a proteomických přístupů plánujeme odhalit adaptivní mechanismy, které vedou k obnovení metabolické homeostázy. Cílem je odhalení klíčových metabolitů, případně metabolických drah, které by mohli být využity v terapii.

Investigating the role of Src in osteoclasts

doc. RNDr. Daniel Rösel, Ph.D.

ID 246354



The product of the c-src proto-oncogene, tyrosine kinase Src, is an essential regulator of cellular physiological processes ranging from cell adhesion, migration to mitogenic and anti-apoptotic signaling. Although Src is ubiquitously expressed, targeted disruption of c-src in mice leads to only one major phenotype, osteopetrosis. This results in the excessive accumulation of bone matrix caused by defective osteoclast functions.

The project aims to analyze the role of Src kinase in the physiology of osteoclasts, especially in the formation of sealing zones. Src activity and dynamics in the osteoclast sealing zone will be analyzed in living osteoclasts using our Src-FRET biosensor. CRISPR/Cas9 knock-in strategies will be used to prepare monocytes expressing the Src-FRET biosensor under an endogenous promoter.

Candidate profile:

The PGS candidate should have experience in mammalian cell cultivation techniques and basic fluorescence microscopy. Experience with live-cell microscopy, FRET and/or CRISPR/Cas9 are of further advantage.

Suggested reading:

Koudelková L, Pataki AC, Tolde O, Pavlik V, Nobis M, Gemperle J, Anderson K, Brábek J, Rosel D. Novel FRET-Based Src Biosensor Reveals Mechanisms of Src Activation and Its Dynamics in Focal Adhesions. Cell Chem Biol. 2019 Feb 21;26(2):255-268.e4. doi: 10.1016/j.chembiol.2018.10.024.

PKN3 signaling at the crossroad of Rho signaling and mitochondrial metabolism

doc. RNDr. Daniel Rösel, Ph.D.

ID 246408

PKN kinases belong to family of PKC kinases and are involved predominantly in regulation of cytoskeletal organization as effector proteins of Rho family of small GTPases. Unlike other PKN kinases, PKN3 is physiologically expressed mostly in primary endothelial cells and osteoclasts but is also often overexpressed in cancer cells. Recently, PKN3 was found to be surprisingly enriched in mitochondria. The aim of the project will be to analyze a potential PKN3-mediated crosstalk of Rho signaling and mitochondrial physiology.

The PGS candidate should have experience in mammalian cell cultivation techniques and basic fluorescence microscopy. Experience with live-cell microscopy, and/or CRISPR/Cas9 are of further advantage.

Development of therapeutic exosomes and gene therapy for Diamond Blackfan Anemia (DBA)

doc. RNDr. Radislav Sedláček, Ph.D.

ID 253151

Development of Extracellular vesicle (EV)-based and AAV-based gene therapy of hematopoietic stem cells (HSC) for the treatment of the Diamond-Blackfan Anemia (DBA) model. Development of delivery/therapeutic protocols.

In vitro/ex vivo correction of HSC using genetic approach and site-specific nucleases. In vivo analysis of DBA disease mouse model after the application of the gene therapy.

Conditions of international mobility of researchers: The applicant must not have resided or carried out his or her main activity (work, studies, etc.) in the Czech Republic for more than 12 months in the 36 months immediately before the date of recruitment.

Link to the tender: https://www.img.cas.cz/2022/09/66643-phd-position-in-exosome-mediated-gene-therapy-of-diamond-blackfan-anemia/

Investigating mechanisms of hematopoietic stem cells and their therapy resistance in hematological malignancies - cellular, murine, humanized models

prof. MUDr. Tomáš Stopka, Ph.D.

ID 246112

The maintenance of human health is the result of a dynamic interaction of external and internal forces that include environmental cues, genetic background and its epigenetic realization into various cellular products that give rise to phenotypic expressions. Clonal disorders, such as solid tumors and hematologic malignancies, are the result of alteration of unique homeostatic interactions in stem cells. They involve early chromatin remodeling during differentiation and subsequent involvement of key transcription factors. Thus, chromatin represents a major barrier to the development of genetic variants and deregulated gene expression involved in hematopoietic cell diseases such as leukemia, lymphomas and myeloma. Modelling such phenomena involves intensive genetic research, cloning, development of transgenic models in cell lines or mice and, not least, the development of xenograft implantation of human tumor cells into immunocompromised mice. Testing of newly defined mechanisms includes, among other things, validation studies with inhibitors of newly identified target pathways.

The objectives of the proposed project:

1) to identify the mechanisms involved in the stem cell response to anticancer therapy;

2) to determine how epigenetic mechanisms influence the molecular causes of tumor transformation.

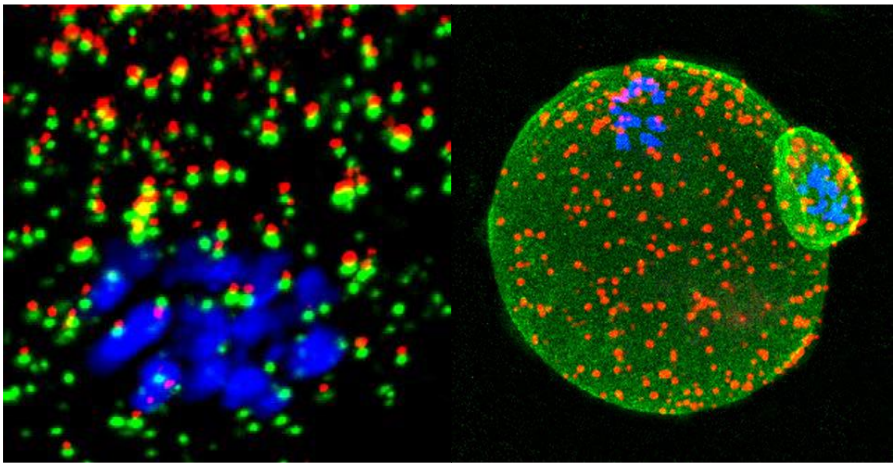
Our research program involves the design of innovative transgenic mouse and cell culture systems and advanced molecular methods, including next-generation genome sequencing, and the development and testing of novel modalities useful in therapy. We are looking for scientifically curious and talented predoctoral and postdoctoral fellows to join the modern laboratory environment in the Czech Republic (BIOCEV) and abroad (IARC) to develop conceptually new projects in the above research areas.

Reprogramming of translation during oogenesis and embryogenesis

Ing. Andrej Šušor, Ph.D.

ID 246705

A subset of maternal transcripts is stored in a dormant state in the oocyte, and timely driven translation of specific mRNAs guides meiotic progression, 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/proteome stabilization and protein synthesis. We recently showed that tightly controlled translational program is essential for proper meiotic maturation, fertilization and subsequent development of the offspring. Thus, understanding the key molecular regulators of translational control is very important. High resolution polysome fractionation and low-input ribosome profiling of mouse oocytes and preimplantation embryos has enabled us to define the translational landscape at an unprecedented level. We systematically and comparatively analyzed the transcriptome, polysome- and nonpolysome-bound RNA profiles of mouse oocytes and early embryos at 2- cell, 8-cell, morula and blastocyst stage. We defined four modes of translational selectivity: i. selective translation of non-abundant mRNAs, ii. active translation of highly expressed mRNAs, iii. translationally suppressed abundant mRNAs, and iv. mRNAs bound by monosomes. We found that translatome positively correlates with transcriptome in oocytes, followed by a markedly different translational control in 2-cell embryos, which is gradually synchronized at the later developmental stages. We identified important novel cellular/embryonic functional regulators that are being utilized and prioritized for translation at every developmental stage accompanied by enigmatic embryonic developmental programming. Together, these data reveal a unique, stage-specific, temporal regulation o translation that accompanies oocyte and preimplantation development. This project will unravel new mechanisms of regulation of development of mammalian egg and preimplantation embryo, including human.

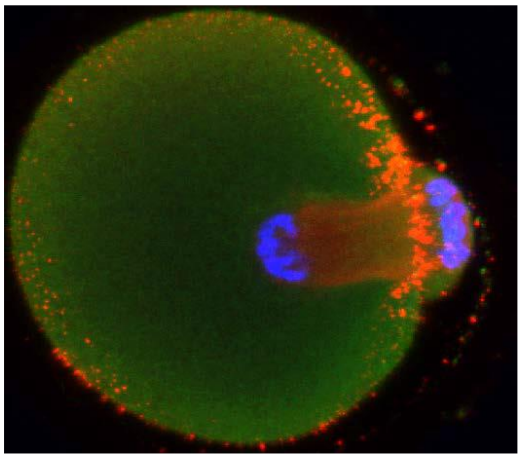


Temporal regulation of translation during oogenesis and embryogenesis

Ing. Andrej Šušor, Ph.D.

ID 257741

Translational control of specific mRNAs is a widely used mechanism of gene regulation. In particular, the oocyte requires precisely controlled translation of maternal mRNAs to coordinate meiosis and embryonic development when transcription is ceased. Our results show significant perturbations of translation during oocyte meiosis and embryonic development. Activation and deactivation of various translation factors go hand in hand with mRNA metabolism and protein synthesis. Based on our data, we hypothesize that translational regulation of specific mRNAs is an important co-regulatory mechanism that ensures the precise expression of proteins responsible for the successful regulation of cytokinesis. The goal of this project is to understand the newly observed phenomenon that shows significant changes in the regulation of protein expression at specific time points. This project will uncover new regulatory mechanisms in mammalian egg and early embryo development.



Therapeutic influence of Sertoli cells on the effects of oxidative stress in germ cells

RNDr. Tereza Tlapáková, Ph.D.

ID 254388

Infertility and fertility disorders are currently much-discussed issues. They affect 8-12 % of couples worldwide, of which approximately 40-50 % are caused by problems on the man's side, mainly the inability to produce enough healthy sperm. Research has confirmed the key role of oxidative stress in the etiology of male infertility, as 30-80 % of infertile men have elevated levels of reactive oxygen species (ROS) in their seminal fluid. A physiological amount of ROS is necessary for proper spermatogenesis. ROS plays an important role in, for example, signal transduction or sperm maturation. However, if they increase above the physiological limit, they can cause damage to the germ cell lines, thus disrupting the function of sperm and thereby adversely affecting fertility. It has been shown that some types of infections can cause an increase in testicular ROS levels, testicular inflammation, and disruption of the blood-testis barrier in the seminiferous tubules, leading to activation of the immune system and damage to testicular tissue and germ cells. These infections include epididymitis, orchitis, mumps, and some sexually transmitted infections (Chlamydia trachomatis, Mycoplasma genitalium, hepatitis B virus). The result can be a partial or complete cessation of spermatogenesis or sterility.

In recent research, Sertoli cells can transfer mitochondria to damaged immune cells and thereby influence their survival. Sertoli cells are highly specialized cells located on the basement membrane of the seminiferous tubules of mammalian testes. They perform many functions necessary for proper spermatogenesis, and their proper organization and development are the basis of fertility. Sertoli cells provide mechanical support, protection, and nutrition for the developing germ cells and also form the blood-testicular barrier through tight junctions between neighbouring cells, making the testis an immunologically privileged organ.

This project will aim to find out whether the transplantation of Sertoli cells after inducing testicular inflammation can prevent the apoptosis of germ cells damaged by oxidative stress and thereby preserve spermatogenesis. Furthermore, it will be observed whether Sertoli cells are capable of transferring mitochondria to damaged germ cells and thereby eliminating the unwanted production of ROS by defective mitochondria. In the next part, this project will deal with the creation and characterization of 3D testicular organoids in vitro in humans, which will subsequently be used for a more detailed study of spermatogenesis and research into the possibilities of restoring male fertility after testicular inflammation.

Degradation of maternal proteins during preimplantation development of mammals and its impact on embryonic genome activation

Mgr. Tereza Toralová, Ph.D.

ID 257421

At the beginning of the preimplantation development of mammals all the mRNAs and proteins are of maternal origin. At this stage, embryo is transcriptionally inactive, the embryonic genome is activated during the development. The maternal mRNAs are gradually degraded before the embryonic genome activation. Nevertheless, there is not much information concerning maternal protein degradation. To find proteins whose degradation is necessary for normal course of further development, proteomic analysis of bovine oocytes and embryos is currently being performed in our laboratory. Candidate proteins will be identified and their function during preimplantation development will be thoroughly analysed. It has been found that degradation of some proteins is crucial for activation of embryonic genome and slowdown of cell division. We will focus especially on overexpression of the selected proteins and its influence on embryonic genome activation. The overexpression will be performed using microinjection of fluorescently labelled mRNA into oocytes and embryos. A wide of range of methods will be used for further analysis based on the function of the selected protein (quantitative RT-PCR, time-lapse imaging, confocal microscopy and others). Our model organism is cattle. The advantage of this model is primarily the similarity of the pre-implantation development of cattle and humans, and thus the possibility of transferability of the obtained data to human medicine. At the same time, the obtained data will be verified in other mammalian species to gain an overview of the extent to which the degradation of maternal proteins during early embryonic development is species-specific.

Protein complexes in nucleosomal context

doc. Ing. Václav Veverka, Ph.D.

ID 255480

Eukaryotic gene transcription is regulated by modular protein-protein interaction networks. The aim of the doctoral project will be structural characterization of these complexes in the context of nucleosomes using a combination of structure biology techniques (cryo-electron microscopy, NMR spectroscopy or X-ray crystallography).

The tubulin code in tardigrades

Ing. Stanislav Vinopal, Ph.D.

ID 257855

Tardigrades are microscopic invertebrates with an extraordinary ability to withstand harsh environmental conditions such as drought, low temperatures, and radiation. They achieve this by undergoing morphological transformation into a state of cryptobiosis with an almost negligible metabolic rate. They can revert to their normal state when the conditions are favorable. However, our comprehension of the mechanisms underlying these processes, how they are regulated, and, more importantly, what makes them reversible at the molecular level is limited.

The goal of this dissertation is to investigate the tubulin code in tardigrades, which corresponds to the composition of the microtubule cytoskeleton. Namely, to the expression of particular tubulin isoforms, their post-translational modifications (PTMs), and the PTM enzymes that catalyze them. According to our preliminary findings, tardigrades have a large fraction of particular tubulin isoforms that cannot be affected by certain PTMs, which may have a substantial impact on their microtubule dynamics and stability. As a result, tardigrades' unique tubulin code may represent fascinating adaptation to extraordinary environmental conditions.

To explore this further, we will search for tardigrade homologues of PTM enzymes and conduct phylogenetic analyses based on their different levels of resistance to adverse environmental conditions, particularly desiccation. In collaboration with Dr. Pavel Vopálenský (ÚOCHB, Prague), we will establish an in situ hybridization technique to study tissue-specific expression of tubulin isoforms and PTM enzymes during ontogenetic development and cryptobiosis in tardigrades. We will also examine tubulin PTMs in tardigrades using immunofluorescence.

The coding sequences of identified PTM enzyme homologues will be isolated from selected tardigrade species and functionally validated by expression in mammalian cells. This approach will enable us to express tardigrade tubulin isoforms and co-express them with tardigrade PTM enzymes. Our preliminary findings suggest that some tardigrade tubulins can also be modified by mammalian PTM enzymes. Moreover, we can down-regulate the expression of selected endogenous mammalian PTM enzymes by RNAi, if necessary. If our results reveal unique properties of tardigrade microtubules, we can purify selected tardigrade tubulin isoforms and analyze their biophysical characteristics in vitro in collaboration with the laboratory of Dr. Zdeněk Lánský (BIOCEV, Vestec).

Finally, we will carry out experiments on the expression of nucleic acids or transfection of ribonucleoproteins in tardigrade embryos and adults using modified electroporation and microinjection techniques. If these experiments fail, the study's success would not be jeopardized; however, if successful, they have the potential to increase the study's significance.

The outcomes of this study may greatly enhance our understanding of how cellular machinery adapts to extreme environmental conditions.

Recombinant preparation of mannose-6-phosphate/IGF2 receptor constructs

RNDr. Lenka Žáková, Ph.D.

ID 258855

The growth factor IGF2 has now been demonstrated to have an important role in memory enhancement and consolidation. The effect of IGF2 in improving cognitive function is rapid and sustained and is mediated by the large transmembrane receptor for mannose-6-phosphate/IGF2 (M6P/IGF2R). In addition, increased IGF2 expression plays an important role in the development of certain tumors, and M6P/IGF2R may play a role as a tumor suppressor. Our project is aimed at recombinantly preparing M6P/IGF2R and its variously sized constructs to help investigate the interaction of IGF2 and M6P. The variously sized constructs may contain one or more binding sites for IGF2, M6P. The aim of the project is to elucidate the physiological significance of the interaction of IGF2 and M6P with the M6P receptor using analogues of novel M6P/IGF2 receptor ligands, which play an important role in suppressing tumour growth as well as improving memory and cognitive function.