Developmental and Cell Biology Board

The list of topics and supervisors who offer projects to applicants in the academic year 2021/2022

Phenotypic Studies of the Usher Disease Minipig Model

MUDr. Taras Ardan, Ph.D.

234430

Usher syndrome (USH) is the monogenetically inherited disease characterized by partial or total hearing loss and vision loss that worsens over time. Classified as ciliopathy, it affects ciliary structures in both the inner ear and the retina and 12 genes are assigned to three USH forms where USH1 is the most severe of them. The first pig model for USH was made by manipulating the harmonin-encoding USH1C gene. The study will be focused on the phenotype characterization of the porcine USH1C model using histological, biochemical (RT-PCR, Western blot) and electrophysiological (mfERG, audiometry) examinations of the affected retina and cochlear hair cells.

Influence of 3D nanoscale organization of cellular components on their function

Mgr. Aleš Benda, Ph.D.

ID 235613

The function of most cellular mechanisms is closely related to local spatial organization of the cell, to its 3D ultrastructure. For understanding the principles of different cellular mechanisms it is not enough to know the average chemical composition, mostly simplified to the presence of particular proteins, it is crucial to visualize the 3D spatial heterogeneity which hugely impacts the processes taking place.

The aim of the project is to correlate function and 3D-ultrastructure for selected biological relevant questions by means of correlative 3D light and electron microscopy. The first goal is to contribute to unravelling the role of invadopodia of cancer cells during cell invasion on model 3D systems, the second goal is to characterize influence of various coatings on plasma membrane protrusions of cell monolayers and the third is to quantify nucleolar fibrillar center/dense fibrilar component (FC/DFC) units with relation to replication, transcription and early processing activity at various stages of cell cycle.

The work will include heavy usage of advanced microscopy techniques, especially FIB-SEM, TEM-tomography, confocal, two-photon and super-resolution microscopy and 3D label free holotomography. A key part will be image data processing, including, but not limited to, denoising and deconvolution, image registration, segmentation, volumetric analysis and more.

The role of gamma-tubulin in chromatin organization at nuclear periphery in response to the heat stress.

doc. RNDr. Pavla Binarová, CSc.

ID 231698

The architecture of eukaryotic nuclei contributes to the regulation of gene functions. Our previous work provides evidence on ability of plant and animal gamma-tubulin to assemble into filaments and on the nuclear functions of g-tubulin including its repressive function with E2F transcription factors. Plant lamin-like proteins CRWNs with function in organizing repressed chromatin at the nuclear periphery are proposed to be involved in abiotic stress response. Our indication of CRWN and polycomb complex protein PWO1 with g-tubulin, as well as our preliminary data on changes of g-tubulin localization with silenced chromatin at the nuclear periphery after heat stress, indicated involvement of g-tubulin in heat stress response. The aim of this project is to get indication on g-tubulins role in epigenetic regulation of pericentromeric chromatin under heat stress. We will analyse the effect of g-tubulin silencing amiRNA to transcriptional activation of silenced chromatin with and without heat stress using methodological approaches including chromatin immunoprecipitation ChIP, protein interaction studies, super resolution microscopy, 3D analyses. Our data will contribute to the knowledge of nuclei organization under abiotic stress.

How epigenetic modulators influence phenotypic bi-stability

RNDr. Petr Bartůněk, CSc.

ID 234362

A dominant ENU mutagenesis screen in mice, conducted in the laboratory of Prof. Emma Whitelaw (Australia) has been quite successful in identifying genes which modify epigenetic state. Preliminary analysis of one of the earliest identified mutants, MommeD3, uncovered very unusual and unique phenotypic features in homozygous embryos, including biphenism, imprinting defects, neural degeneration and profound anemia. At that time, the underlying genetic point mutation could not be identified. More recently, taking advantage of next generation sequencing approaches, we have been able to revisit this project and successfully identify the causative mutation. Combining formal description of the embryonic MommeD3 phenotype with global analyses of RNA expression and DNA methylation, the overall aim of the project will be to link the observed stochastic bi-stable embryonic phenotype with potential alterations in genomic imprinting networks.

Neuronal growth cone pathfinding on structured surfaces

Marcus Braun, Ph.D.

ID 231225

During organismal development, growth cones at the axonal ends of neurons navigate a three dimensional maize of paths and obstacles to form the complex network of the nervous system. The cytoskeletal mechanics underpinning growth cone movements are largely unknown. Here, we will employ cultured neurons on structured surfaces to test the motion of growth cones encountering obstacles and choosing different paths. We will image cell movement through artificial maizes and correlate it with the dynamics of the intracellular actin-filament and microtubule network, with a special emphasis on the the positions and dynamics of key filament crosslinkers. This approach will allow us to determine how the cytoskeletal network drives pathfinding in response to spatial confinement of the environment.

The impact of stress on receptor nanotopography on T cells

Mgr. Marek Cebecauer, Ph.D.

ID 234674

The accumulation of critical receptors at the tips of membrane protrusions of unstimulated B and T cells was recently discovered by our group and others (refs.). Such nanoscopic organisation of receptors is key for regulation of signalling events controlling complex immune responses in mammals. In this project, we will study how stress factors (e.g., temperature or oxidative burst) affect three-dimensional organisation of receptors (TCR, CD2, CD4, CD5, CD6) on T-cell surface and their associated effector molecules (e.g., kinases and adaptor molecules) before interaction with stimulating targets. This research will help to understand responsivity/function of T cells in the inflammatory sites, which are often referred to as crucial sites linked to the pathology of autoimmune diseases.

The work will include imaging of receptors and associated effector molecules on the surface of stressed T cells. The imaging approaches will include a panel of advanced fluorescence and super-resolution microscopy techniques, which will be complemented by flow cytometry and some basic biochemistry methods to determine the impact of stress on T-cell function.

Identification and role of proteins involved in the metabolism of genotoxic RNA: DNA hybrids

RNDr. Jana Dobrovolná, Ph.D.

ID 234653

Kolize replikačních vidlic DNA s probíhající transkripcí představují významný a doposud ne příliš prozkoumaný zdroj genetické nestability. Tyto kolize mohou být nebezpečné například u prekancerózních buněk, které bývají vystaveny replikačnímu stresu. Replikační stres a kolize mezi transkripčními a replikačními komplexy jsou spojovány se vznikem vysoce genotoxických RNA:DNA hybridů označovaných jako R-smyčky. Přestože nedávné studie ukázaly, že výskyt R smyček v lidském genomu je relativně častým jevem, molekulární mechanismy jejich vzniku a odstraňování nejsou stále jasné. Cílem této dizertační práce bude: i) pomocí rozsáhlé knihovny siRNA identifikovat proteiny ovlivňující množství R-smyček za využití již zavedeného nástroje pro detekci a izolaci R-smyček; ii) ověřit úlohu identifikovaných proteinů v metabolismu R-smyček a v udržení integrity genomu. V projektu budou využity běžné molekulárně-biologické metody jako je např. imunoprecipitace, SDS-PAGE, imunoblot, RNA interference a imunofluorescence v kombinaci s „high-throughput“ fluorescenční mikroskopií. Student podstoupí krátkodobé stáže na Institute of Molecular Cancer Research, Univetrsity of Zurich, Švýcarsko, kde se setká s experty ve studiu oprav DNA a získá zkušenosti s nejnovějšími přístupy v této oblasti.

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

RNDr. Karel Drbal, Ph.D.

ID 212586

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

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

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

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

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

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

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

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

Cell specific CAG expansion in Huntington disease animal models and characterization of molecular changes in pro-onset stage of the disease

Ing. Zdenka Ellederova, Ph.D

ID 234662

The first aim of the study will be to detect somatic instability of the huntingtin gene in different tissues of mouse (zQ175 HD mice) and porcine models of (KI-85Q-HD) Also a new humanized KI HD model, which is being generated, shall be analyzed, when the first animals are born. Molecular methods such as small-pool PCRs with fluorescent tagged primers will be applied to determine CAG repeat mosaicism in selected tissues and cells. Different cell types will be sorted by FACS, MACS or culture methods from tissues showing somatic expansion, namely in neural tissue, blood, kidneys etc..

The second aim will be to recapitulate and modify somatic CAG expansion in primary and established cell lines in vitro. Using siRNA technology selected gene modifiers of HD will be downregulated to enhance or repress this HD specific phenotype.

The third aimwill be focused on cell type specific monitoring mRNA and protein expression of mutant huntingtin isoforms in pre-onset state of the HD disease models in vitro.

Role of primary cilia in the hematopoiesis and immunity

Mgr. Martina Huranová, Ph.D.

ID 234785

Primary cilia are small membrane protrusions on the cell surface of most cell types in the human body. The major function of the cilia is the communication between cells in tissues. Genetic defects resulting in cilia dysfunction lead to severe disorders called ciliopathies. In our laboratory, we have shown a previously unappreciated link between cilia and the development of blood cells in the bone marrow and immunity. Our study attracted much attention of the scientific community in the field and opened novel directions for our research.

The aim of this project is to investigate the role of cilia in bone marrow stromal cells in the communication with blood cell precursors. We hypothesize that we will uncover novel cell signalling circuits which are crucial for the regulation of blood cell formation. To achieve this goal, we will employ multiple approaches. We will identify and characterize the ciliated cell subpopulations present in the bone marrow. We will establish mouse models with ablated cilia in a specific bone marrow cell subset and examine the effects on hematopoiesis and immunity. We will explore the role of cilia in various diseases connected with altered blood cell formation, such as leukemia, anemia, immune deficiency by utilizing mouse models and human patients.

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

doc. RNDr. Jan Konvalinka, CSc.

ID 225100

Doktorandka bude vyvíjet nové chemicko biologické nástroje pro kvantifikaci, visualisaci a cílení enzymů důležitých pro patogenesi významných lidských chorob a pro hledání jejich účinných inhibitorů. Ve své práci bude využívat metody, nově vyvinuté ve skupině Dr. Jana Konvalinky na UOCHB AV ČR. Jednou z nich je metoda DIANA pro kvantifikaci proteinů využívající konjugátů inhibitorů těchto enzymů s oligonukleotidy. Vazba je pak detekována a velmi přesně kvantifikována pomocí qPCR. Další metodou jsou synthetickéá mimetika protilátek zvaná iBody, polymerní konjugáty založené na hydroxypropylmethakrylamidovém kopolymeru (HPMA), dekorovaném nízkomolekulárními ligandy příslušného enzymu, které umožňují jeho visualisaci, isolaci a/nebo zacílení.
Modelem, na kterém bude tyto nástroje používat, budou cysteinové proteasy z Porphyromonas gingivalis. Tato bakterie, způsobující záněty dásní pacientů s pardentosou, byla identifikována v mozcích pacientů s Alzheimerovou chorobou a velmi nedávno byla postulována hypothesa o možné etiologické souvislosti patologie Alzheimerovy choroby se zánětem způsobeným P. gingivalis. Cysteinové proteasy P. gingivalis jsou důležitým faktorem pathogenity viru a jejich kvantifikace a identifikace může být důležitým nástrojem pro studium a experimentální terapii Alzheimerovy choroby.
Doktorandka bude v rámci své disertační práce připravovat rekombinantní proteasy z P. gingivalis, purifikovat je a charakterisovat. Navrhne a ověří metody na testování jejich aktivity nebo správného složení (foldingu). Ve spolupráci s medicinálními chemiky na UOCHB navrhne, otestuje a validuje metodu na kvantifikaci těchto enzymů jak in vitro, tak na buněčné úrovni. Dále bude identifikovat jejich nízkomolekulární ligandy, a to mj. i testováním rozsáhlé knihovny sloučenin na UOCHB AV ČR. Bude testovat aktivitu polymerních konjugátů dekorovaných těmito ligandy pomocí biochemických metod (SPR, ELISA) a charakterisovat jejich interakci s cílovými enzymy jak pomocí rekombinantních proteinů, tak i tkáňových řezů experimentálních myších modelů.

Práce bude kryta běžícími granty ve skupině J. Konvalinky na UOCHB AV ČR, zejména finanční podporou Gilead Science and IOCB Research Center Prague (do r. 2023) a grantem Evropské komise ERA-NET JPND na studium souvislosti pathogenese Alzheimoreovy choroby

a paradentosy (“Alzheimer’s disease as a co-morbidity of chronic periodontitis with Porphyromonas gingivalis as a causative link between both diseases; Gums and Brains”) ve spolupráci s laboratořemi v Německu, Dánsku, Polsku a Norsku (2020-2023).

Literatura:
Dominy S.S. et al. (2019) Porphyromonas gingivalis in Alzheimer's disease brains: Evidence for disease causation and treatment with small-molecule inhibitors. Sci Adv. 5 (1): eaau3333, DOI: 10.1126/sciadv.aau3333.
Ashraf G.M. et al. (2018) The Possibility of an Infectious Etiology of Alzheimer Disease. Mol Neurobiol. doi: 10.1007/s12035- 018-1388-y.
Šácha, P. et al. (2016). iBodies: Modular Synthetic Antibody Mimetics Based on Hydrophilic Polymers Decorated with Functional Moieties. Angew. Chem. Int. Ed. Engl. 55, 2356-2360
Navrátil, V. et al. (2017) DNA-linked Inhibitor Antibody Assay (DIANA) for sensitive and selective enzyme detection and inhibitor screening. Nucl. Acids Res. 45 (2), e10 DOI: 10.1093/nar/gkw853

Evolution of eyes: insight from photoreceptors of invertebrate chordate amphioxus

RNDr. Zbyněk Kozmik, CSc.

ID 223147

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

Molecular associations between recurrent mutations and immunoresistant phenotype of leukemia cells

RNDr. Kateřina Kuželová, Ph.D.

ID 234646

Imunitní systém patrně hraje zásadní roli v prevenci vzniku leukémií i při eliminaci tzv. zbytkové choroby - leukemických buněk přežívajících v těle pacienta po chemoterapii. Lidský organismus je často schopen spontánně vyvinout protinádorovou imunitní reakci, ta ale může být řadou různých mechanismů následně utlumena. Schopnost buněk unikat imunitnímu systému může být nedílnou součástí leukemogenní transformace. Aktivace signálních drah zajišťujících neregulovanou buněčnou proliferaci nebo odolnost vůči indukci apoptózy může zároveň vést například k redukci exprese HLA molekul, na kterých jsou T-buňkám prezentovány antigeny, ke snížení prezentace antigenů na HLA molekulách, k expresi inhibičních receptorů (např. PD-L1) nebo k sekreci látek tlumících funkci imunitních buněk (např. Gal-9, IDO, arginása). Cílem disertační práce bude hledání souvislostí mezi nejčastějšími mutacemi, které jsou v leukemických blastech nalézány, a přítomností markerů rezistence vůči imunitní odpovědi. Mezi kandidátní mutované proteiny patří nukleofosmin, kináza FLT3 nebo vybrané epigenetické regulátory u akutní myeloidní leukémie nebo fúzní kináza BCR-ABL u chronické myeloidní leukémie. Aktivita těchto proteinů nebo jejich efektorů bude ovlivňována pomocí inhibitorů, siRNA nebo genových modifikací, následky budou studovány na úrovni proteinové exprese (průtoková cytometrie, western blotting, imunofluorescenční mikroskopie, případně imunoprecipitace) a funkčních buněčných analýz (cytotoxické testy, měření metabolismu). Práce bude prováděna převážně na ustavených leukemických liniích, částečně také na primárních leukemických buňkách.

Myosin-independent contractility of actin networks

RNDr. Zdeněk Lánský, Ph.D.

ID 234425

Kontraktilita aktinových sítí je zásadní při mnoha buněčných procesech, jako je například cytokineze, při které uzavírající se aktinový kruh odděluje dvě dceřiné buňky. Tyto procesy mohou probíhat nezávisle na molekulárním motoru myosinu. Molekulární mechanismy těchto procesů však nejsou dosud zcela jasné. Náplň práce kandidáta bude studium kontraktility aktinových sítí nezávislé na myosinu v rekonstituovaných systémech in vitro. Tento projekt bude zahrnovat expresi a purifikaci vybraných cytoskeletálních proteinů, rekonstituci cytoskeletálních vláken a kvantitativní sledování jejich vzájemných interakcí s rozlišením na úrovni jedné molekuly za použití fluorescenční mikroskopie a optické pinzety. Experimentálně získaná data budou kombinována s matematickým modelováním za účelem vytvoření prediktivního popisu studovaného systému.

Characterization of growth and spontaneous regression of melanoma

Mgr. Helena Kupcová Skalníková, Ph.D.

ID 234632

Cutaneous melanoma is an aggressive skin tumor arising from pigmented cells – melanocytes. Melanoma diagnosed at early stages is mostly easily treatable by surgical excision. However, metastatic melanoma is mostly refractory to conventional therapies. Immune system plays a crucial role in melanoma growth control. Interaction between malignant tumor cells and immune system may lead to tumor destruction and its replacement by fibrous tissue. Currently developed immunotherapies targeting PD-1 or CTLA-4 molecules to boost the immune response significantly improved patient outcomes. In addition, adoptive cell transfer using in vitro propagated and activated T cells, originating from melanoma, promotes tumor rejection. The tumor regression may develop also spontaneously without any treatment and disappearance of the part of the tumor occurs in up to 50 percent of human melanoma cases.

Melanoma-bearing Libechov minipig (MeLiM) is a large animal model of hereditary melanoma. In the majority of the MeLiM piglets, a spontaneous regression of melanocytic loci occurs during the first year of postnatal life. The regression is among others accompanied by skin and bristle depigmentation and changes in hematological profile. Characteristic subpopulation of double positive (CD4+ CD8+) T-lymphocytes expanding during spontaneous regression was detected in MeLiM blood and tumor loci. This subpopulation carried mono-specific CDR3 region of T-cell receptor, which together with its onset during regression suggest that these cells may recognize single (melanoma?) antigen.

The aim of this project is characterization of cells and secreted factors that may participate in regulation of melanoma growth or regression. The project involves study of MeLiM animal samples as well as in vitro cultured pig and human cells. Laboratory techniques include mainly flow cytometry, cell culture, biochemical and proteomic techniques.

Development of capturing techniques for dinucleoside polyphosphate capped RNA

Ing. Hana Macíčková Cahová, Ph.D.

ID 234386

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 brand new class of 5‘caps - dinucleoside polyphosphates (NpnN) in bacteria. So far, we have not identified the particular RNA capped with NpnN. The task of the thesis is to develop selective technique that allows for identification of RNA capped with these caps. We will employ methods of next-generation sequencing such as Illumina or Oxford Nanopores in combination with enzymatic and chemical reactions. After the development of the technique, student will focus on the role of these RNA caps in RNA metabolism, in general.

New molecular mechanisms of genome integrity in human cells

MUDr. Libor Macůrek, Ph.D.

ID 234475

Poškození DNA může vést k rozvoji různých onemocnění včetně rakoviny. Zdravé buňky disponují efektivními kontrolními mechanismy schopnými opravit různé typy poškození DNA a zabránit tak přenosu mutací do dceřiných buněk. Molekulárním základem těchto procesů je aktivace ATM/ATR kináz a E3 ubiquitin ligáz vedoucích k fosforylaci a ubiquitinaci chromatinu v okolí DNA poškození. Posttranslační modifikace jsou rozpoznávány specializovanými vazebnými doménami proteinů a umožňují prostorově i časově kontrolované nasedání proteinů účastnících se opravy DNA. Fosfatáza WIP1 napomáhá včasné terminaci této signální dráhy prostřednictvím defosforylace řady substrátů vázaných na chromatin.

Cílem tohoto projektu je identifikovat nové molekulární mechanismy buněčné odpovědi na poškození DNA nezbytné pro udržení integrity genomu. Především se zaměříme na nově nalezené posttranslační modifikace proteinů DDR dráhy vyskytující se po vystavení buněk genotoxickému stresu. Kandidátní fosforylační místa na proteinech NUMA, SMC1 a TRF2 již byla identifikována prostřednicím proteomického screeningu. Hlavním cílem projektu je určit význam těchto modifikací pro správnou odpověď buňky na poškození DNA. V prvé řadě určíme, které kinázy PI3K-like rodiny jsou zodpovědné za indukci těchto modifikací a po jakých typech DNA poškození. Dále budeme sledovat dynamiku, s jakou se tyto modifikace objeví po poškození a jak dlouho budou přetrvávat. Testován bude předpokládaný vliv WIP1 fosfatázy na odstraňování zmíněných modifikací po opravě DNA poškození. Pro stanovení významu těchto modifikací budeme sledovat dynamiku opravy DNA po depleci proteinů metodami RNAi. Vzhledem k předpokládané toxicitě úplné inaktivace kódujících genů využijeme nové verze CRISPR/Cas9 technologie umožňující cílenou manipulaci jednotlivých bazí. Tímto způsobem připravíme buněčné linie nesoucí substituce fosforylačních míst na Alanin a Aspartát a budeme studovat vliv na opravu poškozené DNA. Oprava dvouvláknových zlomů bude sledována nepřímo prostřednictvím fluorescenční mikroskopie a vyhodnocení množství jaderných fokusů zavedených markerů (např. gH2AX, 53BP1, Rad51). Případné změny morfologie těchto jaderných struktur budou studovány konfokální nebo super-resoluční mikroskopií. Vliv mutací na přežívání buněk bude stanoven prostřednictvím vyhodnocení schopnosti tvořit kolonie případně měřením metabolické aktivity mitochondrií (MTT esej). Obdobným způsobem jako výše zmíněné fosforylace bude rovněž studován význam ubiquitinace chromatinu pro nasedání vybraných proteinů účastnících se opravy DNA.

Tento projekt přispěje k lepšímu porozumění mechanismu ochrany buněk před rozvojem nestability genomu. Konkrétně může objasnit význam cohesinového komplexu a dalších jaderných struktur pro opravu DNA.

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

Mgr. Marie Macůrková, Ph.D.

ID 234618

Clathrin-mediated endocytosis is an important cellular process involved in uptake of molecules into cells, signalling or membrane homeostasis. It is rigorously controlled at several levels. At the plasma membrane the clathrin pit assembly and cargo selection is assisted by numerous adaptor proteins, among them the AP2 adaptor protein complex. In mammalian cells AP2 complex 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 seems to be 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 type. 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 in case of migration, 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.

Deciphering the direct WNT-NOTCH cross-talk in mammals

Mgr. Jan Mašek, Ph.D.

ID 234630

Aim of the proposed project is to define and functionally test the direct Wnt-Notch pathways interactions through the Notch receptor intracellular domain (NICD) in mammals.

The cross-talk between Notch and Wnt/β-catenin signaling became so integral that a joint name Wntch was proposed (1). However, the underlying protein-protein interactions remain largely elusive. Successful applicant will use a combination of molecular biology methods to reveal and modify the amino acids required for interaction of Notch1/Notch2 ICDs with either β-catenin, Gsk-3β, Dvl2/WWP2, Axin or Apc, and test the outcomes on "Canonical" Notch and Wnt signaling. Applicant will mutate the respective binding motifs in either Notch1 or Notch2 genes in vivo using a mouse model to understand their patho/physiological importance in development, adulthood and cancer. In parallel, the project involves the usage of newly developed cell lines in search of novel binding partners, and gene targets using MS-Chip technology.

Student will be encouraged and supported in seeking additional fellowship funding and collaborative projects abroad.

Techniques:confocal microscopy, gene targeting, mouse in vivo experiments, cloning, WB, ISH immunoprecipitation, sc-RNA-seq, MS-Chip.

Challenging the "Non-canonical" signaling potential of Notch ligand Jagged 1 in mammals

Mgr. Jan Mašek, Ph.D.

ID 234629

The project takes advantage of the known Allagile syndrome-causing mutations in J1ICD (1), and recapitulate them in vivo using mouse models to understand their pathophysiological role in development. We will apply the newly developed animal/cell tools to search for novel binding partners of J1ICD, and gene targets using MS-Chip technology. Describing a Notch ligand cis-signaling bears the potential to fundamentally change the field of Notch biology.

Students will be encouraged and supported in seeking additional fellowship funding and collaborative projects abroad.

Techniques: confocal microscopy, gene targeting, mouse in vivo experiments, cloning, WB, ISH immunoprecipitation, sc-RNA-seq, MS-Chip.

Generation and Functional Characterization of hiPSC-derived retina pigmented epithelial cells (RPE)

prof. MVDr. Jan Motlík, DrSc.

ID 231704

The proposed PhD program is oriented to biomedical research till now incurable human disease: the dry form of the age related macular degeneration. Because the incidence of this disease is permanently increasing, orientation of this research is very actual.

In the first part, PhD program will require to test all recent cell culture methods, first of all in vitro culture of RPE cells on the nano-membrane scaffolds. This step will be essential for the correct orientation of RPE cells during transplantation in the subretinal space.The main accent will be given on the complex characterization of hiPSC-derived RPE cells from aspects of morphology, biochemistry and molecular genetics.

The key aspect of the second part will be focused to the efficient and safety cryo-conservation and their preparation for the autologous transplantation. Program will require student's active participation in the team work, which is represented by the Czech (University Hospital Kralovske Vinohrady, Institute of Macromolecular Chemistry, CAS) and foreign (University of Oslo, University of Velencia) partners.

Experiments proposed in the PhD program will be supported by TACR KAPPA grant (TO01000107) which is planned for a period of 2021-2024 and it secures a continuity of the program. Although PhD program will be managed from the Laboratory of cell regeneration and plasticity, IAPG in Libechov, the main laboratory activity will be realized in the Tissue Bank of the Ophthalmologic Clinic, University Hospital Kralovske Vinohrady in Prague.

Molecular mechanisms of pathogenicity in ATP synthase disorders

RNDr. Tomáš Mráček, Ph.D.

211570

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

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

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

Relevant publications:

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

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

Adaptations of cellular metabolism to dysfunction of mitochondrial oxidative phosphorylation

RNDr. Alena Pecinová, Ph.D.

ID 223242

Mitochondria play an important role in ATP production and cellular homeostasis by provision of intermediates for biosynthetic pathways. In humans, the defects of oxidative phosphorylation (OXPHOS) lead to severe mitochondrial diseases, affecting tissues with high energetic demands. In order to maintain ATP and carbon homeostasis, diseased cells need to reconfigure their metabolic pathways (known as metabolic rewiring). Dual genetic origin of OXPHOS enzymes hampers development of primary gene therapy, which triggered intensive research aimed at identification of disease modifying agents able to alleviate metabolic stress. However, the spectrum of metabolic adaptations is very broad depending on type and severity of the defect.

In the current project, unique mammalian models with primary defects in OXPHOS will be studied. The aim is to decipher the adaptive mechanisms that restore metabolic homeostasis and will allow to pinpoint key metabolites or pathways amendable to therapies.

Role of Fam84b in regulation of retinal development and homeostasis

Mgr. Jan Procházka, Ph.D.

ID 234696

Project will be focused on identification of molecular function of newly described gene Fam84b. The phenotype is mostly apparent in retina, where Fam84b deficiency leads to age dependent neurodegeneration with signs of macular degeneration. Based on homology similarities with LRAT family, molecular biology and biochemistry experiments will be designed to prove or disprove involvement of Fam84b in Ras signaling pathway regulation. The methods will be based on protein tagging and localization of protein in cells. BioID proximity ligation assay and co-precipitation proteomics in order to reveal interacting partners. In parallel the Fam84b model will be used as mouse model with age dependent macular degeneration which we will intervene by possible gene therapy vectors designed to interact with revealed dysregulated signaling pathways.

Molecular mechanisms of axon guidance

Mgr. Daniel Rozbeský, Ph.D.

ID 234634

All that we know, and all that we are, comes from the way our neurons are connected. There are about 1011 neurons in our brains, and each neuron makes on average thousand synapses with other neurons. Thus, the human brain contains about 1014 synaptic connections that give rise to our memory, intelligence, speech, movement, sensation or emotion. How are these highly organized neuronal networks correctly established? What molecules are involved in setting-up neuronal connections? What are the neuronal networks underlying specific behaviours?

Our group is focused on molecular mechanisms of axon guidance and neural connectivity. We are particularly interested in semaphorins, the largest families of axon guidance cues which exert repulsive or attractive effects on axon growth cones through interaction with the plexin family of cell surface receptors.

As a PhD student, you will aim to understand how proteoglycans modulate the axon navigation towards their target. You will use protein crystallography and advanced fluorescence microscopy that will be further combined with biophysical and cellular experiments. Furthermore, you will build key oral and written communication skills, techniques for open science, and how to lead collaborative research projects. The project will give you opportunities to present your findings at scientific meetings. Our group has a strong commitment to both personal and professional development, and you will be encouraged to develop your own ideas within the scope of the group's interests and build your academic profile.

The work will be based at a new research centre, Biocev, which houses scientists of international repute in related fields and provides a broad range of frontline structural biology techniques.

You should hold or, be near completion of a master's degree in biochemistry, structural biology, or related discipline, and have hands-on experience in techniques relevant to the project. Particularly useful would be the experience of protein production and purification, plasmids construction, cell culture, protein crystallography or electron microscopy. Good communication and writing skills are essential.

Molecular mechanisms of MICAL signalling in cytoskeletal dynamics

Mgr. Daniel Rozbeský, Ph.D.

ID 232660

MICALs (Molecules Interacting with CasL) are a family of unique signalling molecules that directly bind and disassemble actin filaments and are known to play essential roles in cell processes requiring discrete changes in the cytoskeleton. In a collapsing axon growth cone, MICALs provide a direct link between semaphorins, plexins and the F-actin collapse. Although the field has made enormous advances in understanding MICAL function, our knowledge of the molecular mechanisms of MICAL signalling is still lacking.

As a PhD student, you will aim to understand the molecular mechanisms of MICAL activation and autoinhibition. You will use cryo-electron microscopy and protein crystallography that will be further combined with biophysical and cellular experiments. Furthermore, you will build key oral and written communication skills, techniques for open science, and how to lead collaborative research projects. The project will give you opportunities to present your findings at scientific meetings. Our group has a strong commitment to both personal and professional development, and you will be encouraged to develop your own ideas within the scope of the group's interests and build your academic profile.

The work will be based at a new research centre, Biocev, which houses scientists of international repute in related fields and provides a broad range of frontline structural biology techniques.

You should hold or, be near completion of a master's degree in biochemistry, structural biology, or related discipline, and have hands-on experience in techniques relevant to the project. Particularly useful would be the experience of protein production and purification, plasmids construction, cell culture, protein crystallography or electron microscopy. Good communication and writing skills are essential.

Structural basis of plexin signalling

Mgr. Daniel Rozbeský, Ph.D.

ID 234637

All that we know, and all that we are, comes from the way our neurons are connected. There are about 1011 neurons in our brains, and each neuron makes on average thousand synapses with other neurons. Thus, the human brain contains about 1014 synaptic connections that give rise to our memory, intelligence, speech, movement, sensation or emotion. How are these highly organized neuronal networks correctly established? What molecules are involved in setting-up neuronal connections? What are the neuronal networks underlying specific behaviours?

Semaphorin ligands and their plexin receptors are one of the classical cell guidance factors that play essential roles in cell processes requiring discrete changes in the cytoskeleton. Although the field has made enormous advances in understanding semaphorin function at the level of genetic and cellular experiments, our knowledge of the molecular-level mechanisms of semaphorin signalling is still lacking.

As a PhD student, you will aim to understand structural basis through which the extracellular and cytoplasmic segments of plexin receptors communicate and dissect a long-standing question on how does a signal from outside the cell pass to the cytoplasmic plexin domain. You will use protein crystallography and cryoEM that will be further combined with biophysical and cellular experiments. Furthermore, you will build key oral and written communication skills, techniques for open science, and how to lead collaborative research projects. The project will give you opportunities to present your findings at scientific meetings. Our group has a strong commitment to both personal and professional development, and you will be encouraged to develop your own ideas within the scope of the group's interests and build your academic profile.

The work will be based at a new research centre, Biocev, which houses scientists of international repute in related fields and provides a broad range of frontline structural biology techniques.

You should hold or, be near completion of a master's degree in biochemistry, structural biology, or related discipline, and have hands-on experience in techniques relevant to the project. Particularly useful would be the experience of protein production and purification, plasmids construction, cell culture, protein crystallography or electron microscopy. Good communication and writing skills are essential.

Src signaling in regulation of cellular adhesion and mechanotransduction

doc. RNDr. Daniel Rösel, Ph.D.

ID 223021

Src kinase plays an important role in a multitude of fundamental cellular processes and is often found deregulated in tumors. Despite the scattered nature of the data, growing body of evidence emerges indicating the importance of Src kinase also in mechanotransduction. In this context, Src, in tight cooperation with primary sensors and the cytoskeleton, functions as an effector molecule necessary for transformation of mechanical stimuli into biochemical outputs executing cellular response and adaptation to mechanical cues.

The project aims to analyze the role of Src-p130Cas-Crk signaling axis in cellular adhesion and mechanotransduction. Within the project the role of Src will be analyzed within the podosome-type adhesion structures and mechanosensory properties of Src and p130Cas will be evaluated taking advantage of in lab prepared Src and p130Cas biosensors. Further, mutagenesis and newly designed specific inhibitors will be used to affect the Src-p130Cas-Crk signaling axis and thus the invasive properties of cancer cells.

Plasticity of cancer cells invasiveness and its targeting by migrastatic drugs

doc. RNDr. Daniel Rösel, Ph.D.

ID 212240

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 determination of p130Cas role in mechanics of Cell-ECM mechanosensing

doc. RNDr. Daniel Rösel, Ph.D.

217751

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

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

Introduction of a Mollusc model system into molecular biology

prof. Mgr. Petr Svoboda, Ph.D.

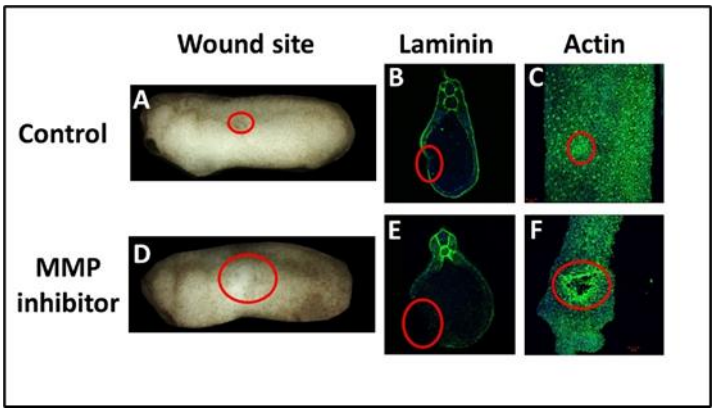
ID 235955

Aim of the disseration thesis is an introduction of a mollusc model system for functional genomics. This thesis develop one or two selected mollusc model systems into an experimental model in the lab, which will include 1) development of basic protocols for maintaining, breeding, and phenotyping (development, anatomy, histology), 2) characterization and improved annoation of the genome, with focus on annoation of genes expressed in the germline (including long non-coding RNAs and identification of small RNA sources), and 3) establishment of transgenesis targeted mutagenesis and analysis of biological roles of germline-specific small RNA pathways

Gene expression regulation during early and late phases of embryonic wound healing

Mgr. Radek Šindelka, Ph.D.

234451



Wound healing is a complex and still elusive process. One of the objectives within the field of animal and human medicine is to heal wounds without scars and to effectively replace defective parts of the body. Mammals have the capability of scarless healing, but it is lost after birth and is substituted by scarred healing. Contrastingly, animals such as fishes and amphibians are able to heal without scar formation and completely regenerate a variety of organs even as adults. We recently revealed an important factors for embryonic healing in early (AP1 transcription factors) and late phases (matrix metalloproteinases). We will study signaling pathways and cell types that are responsible for healing and processes that are dependent on these molecules. We will apply a variety of methods ranging from single cell analysis to functional assays using loss/gain of functions and phenotype characterization. We will utilize frog and fish embryo models. Our goal is to uncover mechanisms behind embryonic wound healing and provide clues for improvement of regeneration and healing capabilities.

Molecular Mechanisms of Cancer Resistance: The Role of Mitochondria

Mgr. Jaroslav Truksa, Ph.D.

ID 234746

The project will address the role of mitochondria and their metabolism in cancer resistance as it has become evident that mitochondria play an important role in the process of carcinogenesis and could also participate in the resistance to treatment.

The main aim of this project is to delineate the role of metabolic alterations coupled with mitochondria that participate in the acquisition and/or maintenance of cancer resistance. The role of experimental targets will be tested by gain of function (overexpression) and loss of function (knock-out, CRISPR/Cas system) studies. Genetically modified cell lines will then be tested by live cell imaging using Etaluma720 enabling simultaneous determination of viable and dead cells in real time. Furthermore, we will assess the impact of such modifications on resistance of these cell lines to cell death induction. We also plan to investigate the role of posttranslational regulatory sites of the prospective targets by directed mutagenesis that would either result in loss of function or lead to constitutively active or inactive mutants.

A second aim of the project is to outline the role of lipids in cancer resistance with main focus on cardiolipin and its metabolism. The exact role of cardiolipin in the context of cancer resistance has not been described in detail. Cardiolipin is a specific mitochondrial lipid that enables proper assembly of the respiratory supercomplexes, thus leading to efficient functioning of the OXPHOS inside mitochondria. Recently, it has been shown that the mitochondrial functions are not only regulated by the quantity of this lipid, but also by its quality and the composition of its acyl chains and their oxidation, which greatly affects its biological activity.

The experimental model that will primarily be explored is the model of tamoxifen resistant breast cancer cells due to its high clinical relevance and the fact that it is routinely used in the laboratory.

Targeting hematological malignancies using fragment-based drug design

Ing. Václav Veverka, Ph.D.

ID 212077

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

Role of normal and mutant huntingtin during neural development.

Mgr. Petr Vodička, Ph.D.

234639

Huntington disease (HD) is a hereditary neurodegenerative disorder caused by an expansion of CAG tract in huntingtin (HTT) gene, leading to expression of over 36 glutamines in mutant huntingtin (mHTT) protein. HTT mutation leads to both the loss of function as well as toxic gain of function phenotypes. Gene therapies focused on total HTT lowering are currently in preclinical and clinical testing. As HTT plays important, but not yet fully elucidated role in the cell differentiation and embryo development, this may have important consequences for safety of HTT lowering therapies. The aim of this project is to study fetal brain development in presence of mHTT using unique knock-in minipig expressing HTT with 86Q in vivo. Human HD iPSC lines, normal HTT iPSC lines and possibly HTT null lines will be used as model of neural cell differentiation in vitro. Methods will include immunohistology, proteomics, tissue culture and live cell microscopy.