OR Vývojové a buněčné biologie

Vypsané doktorské práce pro akademický rok 2020/2021- II. kolo přijímacího řízení

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.

Neuronal growth cone pathfinding on structured surfaces

Marcus Braun, Ph.D.

ID 231225

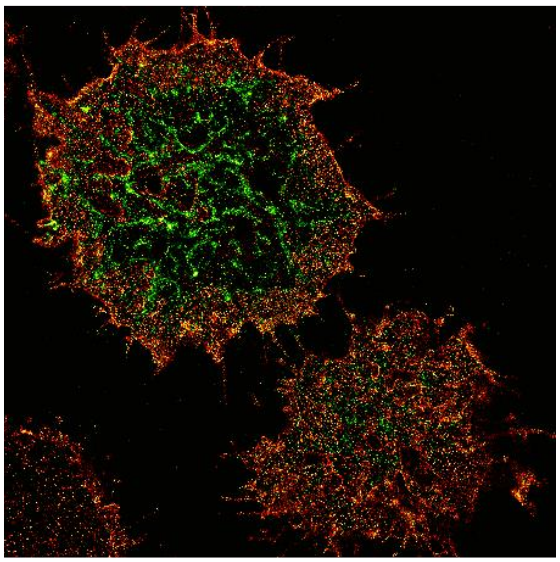
During organismal development, growth cones at the axonal ends of neurons navigate a three dimensional maize of paths and obstacles to form the complex network of the nervous system. The cytoskeletal mechanics underpinning growth cone movements are largely unknown. Here, we will employ cultured neurons 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.

Unravelling molecular surface nanotopography of immune cells by advanced microscopy

Mgr. Marek Cebecauer, Ph.D.

ID 212224

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



Identification and role of proteins associated with genotoxic RNA:DNA hybrids

RNDr. Jana Dobrovolná, Ph.D.

ID 231715

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.

Changes in cell cycle regulation, mitochondrial and antioxidant mechanism of protection of cells expressing mutated huntingtin

Ing. Zdenka Ellederová Ph.D.

ID 231828

The aim of this work will be to elucidate the molecular changes related to cell cycle regulation, metabolism and apoptosis in Huntington's disease.

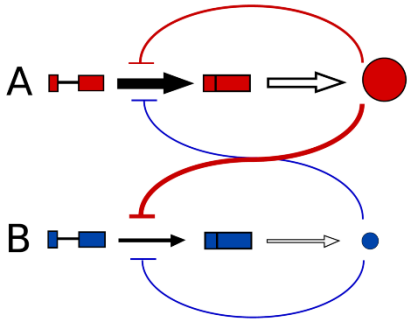
Preliminary results have showed gradual accumulation of oxidative stress and aberrant regulation of the G2 / M phase in cells expressing mutated huntingtin (mHTT). mHTT has been shown to bind to ATM, a serine / threonine kinase that is activated by double-stranded DNA breaks. It phosphorylates several key proteins that initiate cell cycle arrest, DNA repair, or apoptosis.

The student will study and compare cell cycle regulation, metabolism and apoptosis in primary cell cultures isolated from the mouse (HD-Q175) and mini-pig (KI-85Q) model for Huntington's disease as well as in human induced pluripotent stem cells or primary cells (fibroblasts or blood cells) isolated from human patients. The mHTT binding to the key components of the cell cycle together with mHTT influence on its regulation will be studied.

The roles of RNA structures in the regulation of splicing

doc. RNDr. Petr Folk, Ph.D. (folk@natur.cuni.cz)

ID 222547



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

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

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

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2. M. B. Warf, J. A. Berglund, Trends Biochem. Sci. 35, 169–78 (2010).
3. S. Rouskin et al., Nature. 505, 701–5 (2014).
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Understanding the factors critical for successful paternal genome remodelling

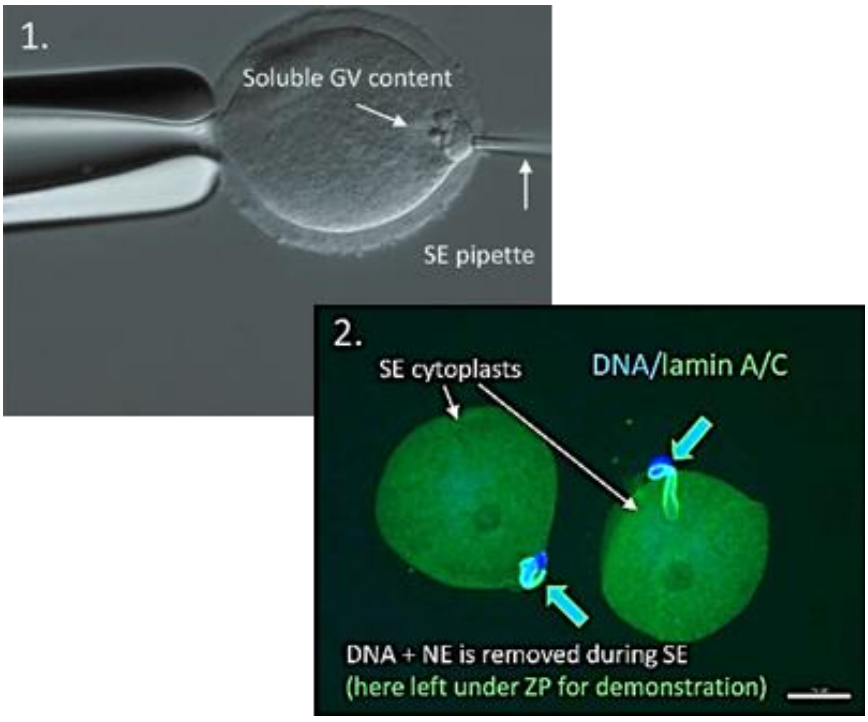
Mgr. Helena Fulková, Ph.D.

ID 223126

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

Suggested reading:

https://www.ncbi.nlm.nih.gov/pubmed/?term=fulka+h



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).

Characterization of growth and spontaneous regression of melanoma

Mgr. Helena Kupcová Skalníková, Ph.D.

ID 231829

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, later stages are mostly refractory to conventional therapies. Currently developed immunotherapies significantly improved patient outcomes. Interestingly, in up to 50 percent of human melanoma cases, partial spontaneous regression, i.e. disappearance of the part of the tumor in absence of any treatment, occurs. Immune system plays a crucial role in melanoma growth control and melanoma represents an immunogenic tumor capable of activating or modulating the immune response.

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 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 T-cell receptor, which suggest that these cells may control tumor growth and regression.

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 human cells. Laboratory techniques include mainly flow cytometry, cell culture, biochemical and proteomic techniques.

Regulatory roles of the microtubule envelope

RNDr. Zdeněk Lánský, Ph.D.

ID 222732

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

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

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

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

Dinukleosid polyfosfátové RNA čepičky v buňkách nespecifického imunitního systému

Ing. Hana Macíčková Cahová, Ph.D.

ID 223131

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

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.

ID 211570

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

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

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

Relevant publications:

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

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

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

RNDr. Alena Pecinová, Ph.D.

ID 223242

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

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

Molecular mechanisms of MICAL signalling in cytoskeletal dynamics

Mgr. Daniel Rozbeský, Ph.D

ID 232660

The overarching aim of this proposal is to understand molecular mechanisms underlying MICAL signalling. 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. Although the field has made enormous advances in understanding MICAL function, our knowledge of the molecular mechanisms of MICAL signalling is still lacking. The aim of the project is to address three fundamental questions:

(1) How does MICAL precisely turn its activity on and off?

(2) How does a signal from outside the cell pass to cytoplasmic MICAL?

(3) How does MICAL activity sculpt the actin cytoskeleton?

To address these challenges, we will use a hybrid approach integrating X-ray crystallography with single-particle cryo-electron microscopy and cryo-electron tomography. This approach will be further combined with cellular experiments in neurons and transgenic flies. Our findings will reveal fundamental principles of MICAL signalling, which can pave the way towards the treatment of MICAL associated neurological disorders.

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.

Src signaling in regulation of cellular adhesion and mechanotransduction

doc. RNDr. Daniel Rösel, Ph.D.

ID 223021

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

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

doc. RNDr. Daniel Rösel, Ph.D.

ID 217751

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

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

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

prof. Ing. Peter Šebo, CSc.

ID 213446

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

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

prof. Ing. Peter Šebo, CSc.

ID 223428

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Targeting hematological malignancies using fragment-based drug design

Ing. Václav Veverka, Ph.D.

ID 212077

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

Transient nuclear protein-protein interaction networks

Ing. Václav Veverka, Ph.D.

ID 212076

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

Role of normal and mutant huntingtin during neural development.

Mgr. Petr Vodička, Ph.D.

ID 231806

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.

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

Ing. Tomáš Vomastek, Ph.D.

ID 212178

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

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

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

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

Ing. Tomáš Vomastek, Ph.D.

ID 212181

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

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

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

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

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

Ing. Tomáš Vomastek, Ph.D.

ID 211488

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