Scientific programme of 25th cytoskeletal club

Wednesday, April 19, 2017

15:00-17:00	Registration
17:20-17:30	Welcome and introduction
17:30-18:30	INVITED SPEAKER Expanding the interactome of the focal adhesion protein TES by exploiting its modules with different subcellular localizations **Ampe Christophe** (Ghent University, Belgium)
18:30-18:45	Getting cytoskeletal 25 times: short review of previous meetings
18:45-18:55	Company presentation **KRD**
19:15-9:00	Dinner, welcome party

Thursday, April 20, 2017

Session II	
9:00-9:20	Role of ARP2/3 complex in plant cuticle formation **Cifrová Petra** (Charles University, Prague, Czech Republic)
9:20-9:40	Plant Class I formins - beyond the actin/plasmalemma/cell wall interface **Cvrčková Fatima** (Charles University, Prague, Czech Republic)
9:40-10:00	High molecular forms of γ-tubulin and γ-tubulin complex proteins in Arabidopsis thaliana **Chumová Jana** (Institute of Microbiology, Prague, Czech Republic)
10:00-10:10	Company presentation **Biotech**
10:10-10:20	Company presentation **Merck**
10:20-10:50	Coffee break
Session III	
10:50-11:10	Are cells grown in vitro similar to their in vivo counterparts when it comes to regulation of cytoskeleton? **Anger Martin** (Faculty of Medicine, Masaryk University, Brno, Czech Rep.)
11:10-11:30	Human γ-tubulin isotypes: differential expression during neuronal development and under oxidative stress

	Sulimenko Vadym (Institute of Molecular Genetics, Prague, Czech Republic)
11:30-11:50	Intense electric field affects tubulin conformation: A molecular dynamics study **Cifra Michal** (Institute of Photonics and Electronics, Prague, Czech Republic)
11:50-12:10	Protein diversity in discrete structures at the distal tip of the trypanosome flagellum **Varga Vladimír** (Institute of Molecular Genetics, Prague, Czech Republic)
12:10-12:20	Company presentation **Sipoch**
12:30-14:00	Lunch
Session IV	
14:00-15:00	INVITED SPEAKER Discovering new roles for Src signaling in regulation of endothelial cells **Karginov Andrei** (University of Illinois in Chicago, USA)
15:00-15:20	PAK1 kinase regulates microtubule nucleation in mast cells **Klebanovych Anastasiya** (Inst. of Molecular Genetics, Prague, Czech Rep.)
15:20-15:40	Regulation of epithelial integrity by MAPK/ERK pathway **Čáslavský Josef** (Institute of Microbiology, Prague, Czech Republic)
15:40-16:00	Tyrosine 90 within SH3 domain - a novel site of Src kinase regulation affecting transformingpotential and invasiveness **Koudelková Lenka** (Charles University, Prague, Czech Republic)
16:00-16:10	Company presentation **Olympus**
16:10-16:50	Coffee break
Session V	
16:50-17:50	INVITED SPEAKER Nucleocytoskeletal networks in cardiovascular disease: The role of lamin A and plectin **Osmanagic-Myers Selma** (Univ. of Nat. Resources and Life Sciences, Austria)
17:50-18:10	Cytoarchitecture in plectin-deficient intestinal epithelium **Kalendová Alžběta** (Institute of Molecular Genetics, Prague, Czech Republic)
18:10-18:30	The Role of Plectin in Hepatocellular Carcinoma **Oyman Eyrilmez Gizem** (Inst. of Molecular Genetics, Prague, Czech Rep.)
18:30-18:40	Company presentation **Bio-port: Spectral analysis, new way of flow cytometry**

19:15 Banquet
20:15 Wine-tasting

Friday, April 20, 2017

Session VI	
9:20-9:40	TbPH1: a kinetoplastid-specific pleckstrin homology domain containing kinesin-like protein **Hashimi Hassan** (Inst. of Parasitology, České Budějovice, Czech Republic)
9:40-10:00	Resolving controversy of unusually high refractive index of tubulin **Křivosudský Ondřej** (Inst. of Photonics and Electronics, Prague, Czech Rep.)
10:00-10:20	Analysis of spatial segregation of the eIF3a mutant in Saccharomyces cerevisiae **Senohrábková Lenka** (Institute of Microbiology, Prague, Czech Republic)
10:20-10:40	Diverse subcellular localization of selected plant Class II formins **Šliková Eva** (Charles University, Prague, Czech Republic)
10:40-11:00	Coffee break

Session VII	
11:00-11:20	Membrane attachment of WASH complex **Humhalová Tereza** (Charles University, Prague, Czech Republic)
11:20-11:40	Single-molecule study of cytoskeletal cross-linkers involved in cytokinesis **Kučera Ondřej** (Institute of Biotechnology, Vestec, Czech Republic)
11:40-11:50	Concluding remarks

12:00 Lunch

Expanding the interactome of the focal adhesion protein TES by exploiting its modules with different subcellular localizations

Stefano Sala[1], Marleen Van Troys[1], Sandrine Medves[2,4], Marie Catillon[2], Evy Timmerman[1,3], An Staes[1,3], Elisabeth Schaffner-Reckinger[2], Kris Gevaert[1,3], Christophe Ampe[1,*]

[1] Department of Biochemistry, Ghent University, Belgium, [2] Cytoskeleton and Cell Plasticity Lab, Life Sciences Research Unit - FSTC, University of Luxembourg, Luxembourg, [3] VIB Medical Biotechnology Center, Ghent, Belgium [4] Laboratory of Experimental Cancer Research, LIH, Luxembourg, Luxembourg

The tumour suppressor TES belongs to the family of LIM-domain containing proteins. This focal adhesion proteins contains in addition a cysteine rich region and a PET-domain that it shares with LMCD1 and prickle proteins. In view of its potential scaffolding function we investigated the cellular behaviour of GFP-tagged variants that lack one or more of these modules. In general, full length TES shows diffuse cytoplasmic localisation with sparse focal adhesion staining in HeLa cells. By contrast the used variants show distinct localisation either at focal adhesions, stress fibers and/or in the nucleus. From this we predicted that these variants may interact with differential partners. To explore this we employed the novel iMixPro AP-MS technology[1]. In conjunction with the TES variants this mass spectroscopy technique allowed to sample TES proximity partners in a subcellular dependent manner. Next to the expected adhesion protein partners we found partners linking TES to metabolic and immune related pathways[2]. Another unexpected outcome of this research was that TES may also exist as a dimeric form. The interaction of TES with two novel partners: TGFB1I1 (a LIM-domain containing focal adhesion protein) and a short form of the glucocorticoid receptor were validated. Interestingly TGFB1I1 opposes the positive activity of TES in cell spreading.

[1] Eyckerman et al., 2016 J Proteome Res. 15:3929-3937
[2] Sala et al., 2017 J Proteome Res. 16 : in press

Discovering new roles for Src signaling in regulation of endothelial cells

Andrei Karginov.
Department of Pharmacology at the University of Illinois in Chicago

Tyrosine kinase c-Src plays a central role in regulation of endothelial barrier function and angiogenesis. To dissect the role of Src signaling in regulation of endothelial cells we employed a novel protein engineering method that provides tight temporal regulation of Src kinase activity in living cells. Endothelial permeability is regulated at the level of adherens junctions, cell-cell adhesion structures mediated by the transmembrane protein VE cadherin. Current model suggests that Src is a negative regulator of endothelial barrier stimulating disassembly of adherens junctions through phosphorylation of VE cadherin. However, our new findings demonstrate that activation of Src can also stimulate enhancement of endothelial barrier. We found that direct activation of Src induces transient reduction in endothelial permeability. This barrier enhancing effect is accompanied by increased formation of structurally distinct reticular adherens junctions that exhibit reduced permeability. Dissection of Src-mediated signaling revealed that VE cadherin phosphorylation and Src-induced morphodynamic changes play a critical role in the enhancement of endothelial barrier. Thus, our results suggest a novel role for Src activity and VE cadherin phosphorylation in regulation of endothelial permeability. Src is known to stimulate podosomes, multiprotein structures that mediate degradation of extracellular matrix by endothelial cells during angiogenesis. We have identified a novel Src substrate, cytoskeletal protein septin 2, that translocates to podosomes upon Src activation. Furthermore, septin 2 is required for podosome formation and matrix invasion by endothelial cells. Interestingly, downregulation of septin 2 expression does not affect endothelial cell migration and tube formation, suggesting a very specific role at the initial step of angiogenesis.

Nucleocytoskeletal networks in cardiovascular disease: The role of lamin A and plectin

Selma Osmanagic-Myers
Department of Biotechnology, BOKU - University of Natural Resources and Life Sciences, Vienna, Austria

Atherosclerosis or hardening of arteries is mainly characterized by increased depositions of fat and extracellular matrix (collagen) eventually causing cardiac defects and death due to myocardial infarction or stroke. Cardiovascular disease is the leading cause of death in the modern society. A known risk factor for cardiovascular disease development is endothelial dysfunction, which is potentiated by blood flow disturbances occurring at regions of arterial branching or by endothelial intrinsic changes altering the ability of cells to sense forces exerted by blood flow over their surface (mechanosensing). Mechanosensing depends mainly on the structural elements of a cell, cytoskeleton and partly also nucleoskeleton. Thus, recent research has generated increasing interest in identifying molecules mediating this mechanosensing response with an ability to modulate the atherogenic response of endothelial cells.

We studied molecular mechanisms of mechanosensing mediated by cytoskeletal and nucleoskeletal proteins in endothelial cells and causes leading to endothelial dysfunction. In this respect we used two endothelium-restricted conditional mouse model systems: plectin-deficient- (P0) and Hutchinson-Gilford progeria syndrome (HGPS) mice (Prog-Tg), expressing mutated lamin A (progerin). For both P0 and Prog-Tg systems, we showed impaired mechanoresponse and endothelial dysfunction in vitro and in vivo, providing novel molecular links for the respective endothelial dysfunction. Furthermore, we could show that actin and vimentin filament cross-talk mediated by the cytoskeletal linker protein plectin plays an essential role for the organization of endothelial cell-cell junctions and vascular barrier integrity. On the other hand, progerin accumulation in endothelial cells disturbed nucleo-cytoskeletal coupling potentiating atherogenic changes through defective cell mechanoresponse and deregulation of factors promoting atherogenesis (in particular Myocardin-Related Transcription Factor A). Atherogenic response could be verified in vivo showing increased collagen depositions surrounding coronary arteries with cardiac fibrosis and hypertrophy in Prog-Tg animals. Altogether, we show for the first time that mutations in plectin and progerin lead to endothelial dysfunction and in case of Prog-Tg mice provide a molecular basis for the severe cardiovascular pathology in HGPS patients. These data unscramble molecular mechanisms through which structural intrinsic changes of endothelial cells potentiate atherogenic events, providing insight into genetic predispositions to endothelial dysfunction and cardiovascular disease.

Role of ARP2/3 complex in plant cuticle formation

Cifrová P., Martínek J., Soukup A., Schwarzerová K.
Department of Experimental Plant Biology, Faculty of Sciences, Charles University, Prague, Czech Republic

When animals are threatened by their environment, they can run away. Plants cannot escape from hostile surroundings. Therefore, they have developed many mechanisms that allow them to resist without leaving their position. Plant cuticle and the cell wall represent one of these mechanisms. Plant cuticle is a thin lipid-derived layer deposited by pavement cells on their surface by still largely unknown transport mechanisms. In our work we investigated the involvement of actin cytoskeleton in cuticle formation. The cuticle layer was not altered by treatment of plants with actin cytoskeleton disrupting drug latrunculin B. We have analyzed the cuticle layer in a wide range of mutants with defects in actin associated proteins to found out that the structure of plant cuticle is disrupted in ARP2/3 complex subunits mutants.

Cuticle layer of ARP2/3 subunits mutants and its activator complex mutants is not continuous and contains numerous cracks, even though on the top of cells we measured higher level of cutin content. Defects in cuticle layer are confirmed by permeability experiment, which shows increased permeability of mutant leaves for Toluidine blue dye. It is possible to track discontinuous cuticle between the pavement cells of ARP2/3 complex mutants even before pavement cells lobe formation begins (3day old plants). Our results suggest that Arp2/3-nucleated actin cytoskeleton subpopulation is involved in the deposition of cuticle in plants.

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Plant Class I formins - beyond the actin/plasmalemma/cell wall interface

Cvrčková F., Oulehlová D.
Department of Experimental Plant Biology, Faculty of Sciences, Charles University, Prague, Czech Republic

Formins, or FH2 proteins, are a family of eukaryotic regulators of cytoskeletal dynamics defined by the presence of the conserved FH2 domain, whose dimer can cap and nucleate actin, and which is often preceded by a profilin-binding, Pro-rich FH1 domain. Many formins also participate in the control of microtubule organization.

Angiosperm plants have two formin clades (Class I and Class II) with multiple paralogs. Most Class I formins are transmembrane proteins with the FH1 and FH2 domains on the cytoplasmic side. AtFH1 is the most expressed Class I formin in Arabidopsis, previously shown to reside in the plasmalemma and anchor the actin cytoskeleton to the cell wall when heterologously expressed in tobacco (Martiniere et al 2011, Plant J 66:354). We documented that AtFH1 modulates the dynamics of both actin and microtubules (Rosero et al 2016 Plant Cell Physiol 57:488, Cvrčková and Oulehlová 2017, Plant Methods 13:19), and constructed transgenic Arabidopsis lines expressing GFP-tagged AtFH1 from its native promoter. Its localization varies in a cell type and developmental stage-dependent manner, and the protein is found not only on the plasmalemma (decorating distinct immobile cortical domains in some cell types), but also at several endomembrane compartments.

Besides of their cytoskeleton-related roles, metazoan formins have been recently implicated also in nuclear functioning (Isogai and Innocenti 2016 Biochem Soc Trans 44:1701), and indeed we previously observed plant Class I formin-derived fusion proteins at the nuclear envelope (Cvrčková et al 2015 Int J Mol Sci 16:1).We performed a transcriptome study in Arabidopsis plants impaired in formin function either pharmacologically or by a mutation inactivating AtFH1, and found that such formin depletion has distinct transcriptional consequences that may hint at additional formin roles.

This work has been supported by the GACR 15-02610S project.

High molecular forms of γ-tubulin and γ-tubulin complex proteins in Arabidopsis thaliana

Chumová J., Kourová H., Trögelová L., Volc J., Benada O., Halada P., Daniel G., Binarová P.
Institute of Microbiology, The Czech Academy of Sciences, Prague, Czech Republic

The γ-tubulin complexes (γTuC) of γ-tubulin with γ-tubulin complex proteins (GCPs) are well established in microtubule nucleation in all eukaryotes. In spite of high conservation of γ-tubulin and GCPs there are organism-specific differences in size and composition of γTuC and there is a variety of regulatory and attachment factors recruiting the complexes to sites of microtubule nucleation and regulating their nucleation activity.

We co-immunoprecipitated with γ-tubulin and identified by mass spectrometry GCP2, 3, 4, 5, and 6, NEDD1, and GIP1 from Arabidopsis thaliana extract. The presence of γ-tubulin complexes with GCPs was validated by reciprocal co-immunoprecipitation and by co-fractionation in sucrose gradient and in size-exclusion chromatography. GCP4 and GCP6 co-fractionated with γ-tubulin in fractions > 1MDa. Pull-down assay with in vitro polymerized plant microtubules confirmed presence of γ-tubulin complexes in microtubular fraction, nucleation assay showed that large forms of γ-tubulin were active in microtubule nucleation. In cellular fractions extending in size large γ-tubulin complexes with GCPs we detected γ-tubulin without the presence of GCPs. Transmission electron microscopy and stimulated emission depletion (STED) super-resolution microscopy were employed to characterize the high molecular γ-tubulin forms. The possible functions of γ-tubulin complexes in vivo will be discussed.

This work was supported by Grant Agency of the Czech Republic (P501 15-11657S).

Are cells grown in vitro similar to their in vivo counterparts when it comes to regulation of cytoskeleton?

Anger M.
Faculty of Medicine, Masaryk University, Brno, Czech Republic

Female germs cells maturing in vitro are not fully comparable to the cells obtained from the ovary. Among the most important differences is their ability to be fertilized and to develop further into the embryo. Other differences, which are already known, include the assembly and morphology of the spindle. We focused on functional characterization of spindles from meiosis II in vitro and in vivo cells. Our experiments revealed that in cells grown in vitro, Eg5 molecular motor is more accumulated on the spindle, and they are also more sensitive to the Eg5 inhibitors. We further discovered that the changes are progressively appearing during in vitro culture. Our study therefore revealed important functional difference between assembly of the spindle in in vitro and in vivo culture conditions. We believe that our results are important for cell biology research where the in vitro grown cells are frequently used as a model system. The conclusions from our experiments indicate that the differences between cells in vivo and in vitro are affecting fundamental and essential processes.

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Human γ-tubulin isotypes: differential expression during neuronal development and under oxidative stress

Sulimenko V.[1], Dráberová E.[1], Vinopal S.[1], Sulimenko T.[1], Sládková V.[1], D'Agostino L.[2], Sobol M.[1], Hozák P.[1], Křen L.[3], Katsetos C. D.[2], Dráber Pa.[1]

1 Institute of Molecular Genetics, The Czech Academy of Sciences, Prague, Czech Republic

2 Drexel University, Philadelphia, USA

3 University Hospital, Brno, Czech Republic

γ-Tubulins are highly conserved members of the tubulin superfamily essential for microtubule nucleation. Humans possess two γ-tubulin genes. It is thought that γ-tubulin-1 represents ubiquitous isotype, whereas γ-tubulin-2 is found predominantly in the brain, where it may be endowed with divergent functions beyond microtubule nucleation. The molecular basis of the purported functional differences between γ-tubulins is unknown. Previously we have reported that in mouse cell line γ-tubulin-2 can rescue γ-tubulin-1 knockdown (PLoS ONE, 7: e29919, 2012). Here we present discrimination of human γ-tubulins according to their electrophoretic and immunochemical properties depending on C-terminal regions of the γ-tubulins. Using epitope mapping, we discovered mouse monoclonal antibodies that can discriminate between human γ-tubulin isotypes. Real time quantitative RT-PCR and 2D-PAGE showed that γ-tubulin-1 is the dominant isotype in fetal neurons. Although γ-tubulin-2 accumulates in the adult brain, γ-tubulin-1 remains the major isotype in various brain regions. Localization of γ-tubulin-1 in mature neurons was confirmed by immunohistochemistry and immunofluorescence microscopy on clinical samples and tissue microarrays. Differentiation of SH-SY5Y human neuroblastoma cells by all-trans retinoic acid, or oxidative stress induced by mitochondrial inhibitors, resulted in upregulation of γ-tubulin-2, whereas the expression of γ-tubulin-1 was unchanged. Fractionation experiments and immunoelectron microscopy revealed an association of γ-tubulins with mitochondrial membranes. These data indicate that in the face of predominant γ-tubulin-1 expression, the accumulation of γ-tubulin-2 in mature neurons and neuroblastoma cells during oxidative stress may denote a prosurvival role of γ-tubulin-2 in neurons.

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Intense electric field affects tubulin conformation: A molecular dynamics study

Marracino P.[1], Grosso A.[1], Havelka D.[2], Průša J.[2], Ayoub A. T.[3], Tuszynski J.[3], Liberti M.[1], Apollonio F.[1], Cifra M.[2]

1 Department of Information Engineering, Electronics, and Telecommunications, Sapienza University of Rome, Rome, Italy

2 Institute of Photonics and Electronics, The Czech Academy of Sciences, Prague, Czech Republic

3 Department of Chemistry and Department of Physics, University of Alberta, Edmonton, Alberta, Canada

Function of proteins and interaction between them is primarily based on electric and electrodynamic forces. Charge distribution and fluctuations of the individual atomic groups on proteins generates local electric field that ultimately determines how proteins bind and interact. Possibility of modifying and possibly controlling protein function by external electric field lends itself as a tool for both understanding and controlling biological processes.

Here we demonstrate using molecular dynamics that intense, yet experimentally attainable, electric field affects tubulin dimer conformation within several nanoseconds. We focused on the effects of electric field on the structure and dynamics of C-terminus of beta tubulin. The tail of C-terminus (i) is important for interactions with other proteins such as motor proteins and microtubule severing proteins, (ii) is a major site for mutations and post-translational modifications, (iii) possesses a significant fraction of tubulin electric charge hence it is a natural target of electric field effects.

We found that field with intensity equal or greater than 20 MV/m (200 V / 10 um) causes straightening of beta tubulin C-terminus as quantified by change of the protein dipole moment. The dynamics of the straightening was shown to be intensity dependent. Tubulin starts to unfold (unzip) from the C-termini for intensities larger than 100 MV/m.

Based on our results we believe that electric field can be used to modify tubulin function by acting on C-terminus tail.

Acknowledgments

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Protein diversity in discrete structures at the distal tip of the trypanosome flagellum

Varga V.[1], Moreira-Leite F.[2], Portman N.[2], Váchová H.[1], Gull K.[2]
1 Institute of Molecular Genetics, The Czech Academy of Sciences, Prague, Czech Republic
2 Sir William Dunn School of Pathology, University of Oxford, Oxford, United Kingdom

The evolutionary conserved eukaryotic flagellum (also known as the cilium) has important motility, signalling and sensory functions. The core cytoskeletal element of the organelle, the so-called axoneme, is constructed by addition of material to its distal end, but molecules orchestrating this process are largely unknown. We took advantage of the high experimental tractability of the flagellated parasite Trypanosoma brucei and developed novel immunoprecipitation-based approaches. These enabled us to identify 8 proteins localizing to the flagella connector at the tip of a growing flagellum and 7 proteins localizing to the axonemal capping structure at tips of all flagella. Both structures contain evolutionary conserved proteins (such as molecular motors, kinases) as well as trypanosome specific proteins. Functional studies using RNAi cell lines revealed roles of individual proteins in these complex structures. This is the first step towards understanding this critical, yet rather intractable region of the eukaryotic flagellum.

PAK1 kinase regulates microtubule nucleation in mast cells

Klebanovych A., Sulimenko V., Hájková Z., E. Dráberová, Dráber Pa.
Institute of Molecular Genetics, The Czech Academy of Sciences, Prague, Czech Republic

Mast cells are known to play a crucial role in allergy, as well as in innate and adaptive immune responses. Mouse bone marrow-derived mast cells (BMMCs) express plasma membrane-associated high-affinity IgE receptors (FcεRIs), the aggregation of which by antigen triggers mast cell activation, resulting in degranulation of the pro-inflammatory mediators. Less is known about molecular mechanisms that control microtubule rearrangement during BMMCs activation. Microtubule nucleation is mediated by γ-tubulin that associates with other γ-tubulin complex proteins to form γ-tubulin ring complexes. Previously we have shown that GIT1/βPIX signaling proteins regulate microtubule nucleation in BMMCs (J. Immunol. 194: 4099, 2015). Here we report that serine/threonine p21 protein (Cdc42/Rac)-activated kinase 1 (PAK1) forms complexes with GIT1/βPIX proteins and γ-tubulin. In vitro kinase assays showed that GIT1 serves as a substrate for PAK1. Pull-down experiments determined an interaction of γ-tubulin with the N-terminal domain of GIT1 responsible for its targeting to centrosome. Immunofluorescence microscopy revealed that both GIT1/βPIX signaling proteins and activated PAK1 kinase localized to centrosomes of interphase cells, and that depletion of PAK1 resulted in decreased nucleation of microtubules from centrosomes. The importance of PAK1 for microtubule nucleation was corroborated by inhibition of its kinase activity with IPA-3 inhibitor that led to decrease in nucleation and recruitment of γ-tubulin to centrosome. PAK1 thus represent positive modulator of microtubule nucleation in BMMCs. We propose that PAK1 with GIT1/βPIX signaling proteins represent a novel regulators of microtubules in mast cells.

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Regulation of epithelial integrity by MAPK/ERK pathway

Čáslavský J., Rasl J., Votavová B., Bartoš Š., Teplá B., Petráčková D., Vomastek T.
Institute of Microbiology, The Czech Academy of Sciences, Prague , Czech Republic

Differentiated epithelium is uniformly polarized multicellular cohesive structure defined by the presence of E-cadherin based cell-cell adhesions at lateral membranes and integrin based adhesions at the basal side. Cell-cell and cell-extracellular matrix adhesions are connected to different types of actin filaments that reinforce the epithelial cell shape and stabilize the multicellular polarized phenotype. MAPK/ERK cascade, comprised of protein kinases Raf, MEK and ERK drives breakdown of epithelial integrity in various cell lines, however, the molecular mechanisms how ERK controls this process remain largely unclear. Here we show, that activation of the ERK cascade in MDCK cells growing in isolated islets resulted in the conversion of epithelial cells to autonomously migrating cells. This process involves two discrete sequentially arranged steps characterized by loss of epithelial polarity followed by cell scattering. We identified that ERK utilizes two different substrates, protease calpain and proteinkinase RSK to control these steps. The activity of protease calpain is necessary for loss of epithelial polarity and associated actin remodeling, whereas RSK regulates increased migratory potential and cell scattering. Interestingly, calpain activation by ERK induces remodeling of actin cytoskeleton at the sites of cell-cell contacts and cell periphery. We suggest that calpain controls actin remodeling through upregulation of RND3/RhoE and thus participate in attenuating RhoA signaling. Conversely, RSK primarily induces weakening of cell-cell contacts and affects the expression of promigratory targets. These data suggest that ERK through the activation of distinct substrates regulates different cellular subprograms. Coordinated execution of these subprograms in time is needed to induce scattered highly motile phenotype of epithelial cells.

Tyrosine 90 within SH3 domain - a novel site of Src kinase regulation affecting transforming potential and invasiveness

Koudelková L.
Faculty of Science, Charles University, Prague, Czech Republic

Src kinase is involved in signalling pathways that participate in the control of cell proliferation, differentiation or motility. Src activity is therefore maintained under strict and complex regulation. Kinase conformation and thus activation state is determined by ability of SH2 and SH3 domains to form inhibitory interactions with intramolecular ligands and by phosphorylation status of key tyrosines 416 and 527.

Phosphoproteomic analysis revealed tyrosine 90 as a new Src residue which can be phosphorylated in vivo. It is localised on the binding surface of SH3 domain, where it forms one of the hydrophobic pockets. Based on the expression of phosphomimic and non-phosphorylable variants (SrcY90E, SrcY90F) in mouse fibroblasts, we observed that Y90 phosphorylation elevates Src kinase activity and alters binding of SH3 domain ligands. Moreover cells bearing SrcY90E mutant exhibit increased invasiveness and transformed phenotype.

We suggest that the phosphate group brings a negative charge into the hydrophobic binding surface of SH3 domain, which causes a decrease in the affinity to CD linker and partial activation of Src. Using intramolecular FRET-based Src biosensor, we have developed, we were able to detect corresponding “opening” of the kinase structure after introducing of Y90E mutation. We propose Y90 as a new additional regulator of Src activity.

Cytoarchitecture in plectin-deficient intestinal epithelium

Kalendová A.
Institute of Molecular Genetics, The Czech Academy of Sciences, Prague, Czech Republic

Plectin is a cytolinker that cross-links cytoskeletal filaments and anchors them to junctional complexes. This way plectin confers resilience to mechanical stress and is essential for tissue stability. Mutations in plectin predispose their carriers to epidermolysis bullosa, a disease manifesting in severe skin blistering, muscular dystrophy, stomach obstruction or digestive disorders. Although molecular mechanisms of skin instability have previously been revealed, the etiology of digestive disorders is yet unknown. To study the function of plectin in intestinal epithelium, we generated intestinal-epithelial-cells-specific, conditional Villin-Cre plectin knockout (PlecIEC-KO) mice. Here we show that PlecIEC-KO mice have lower body weight, suffer from persisting diarrhea and have higher intestinal paracellular permeability. Histological inspection of colon revealed increased dettachment of epithelial cells from basement membrane and extensive inflammation. Immunohistochemical and electron microscopy analysis revealed profound changes in cytoskeletal organization and localization of junctional complexes. In conclusion, our data suggest that plectin maintains polarity and architecture of colonic epithelial cells and its loss leads to inefficient epithelial sealing and damage to the colonic epithelial barrier.

The Role of Plectin in Hepatocellular Carcinoma

Oyman Eyrilmez G.[1,2], Jobe N.[2], Rösel D.[2], Gregor M.[1]
1 Institute of Molecular Genetics, The Czech Academy of Sciences, Prague, Czech Republic
2 Department of Cell and Developmental Biology, Faculty of Science, Charles University, Prague, Czech Republic

Plectin, a prototype cytolinker protein of large size (>500 kDa) and versatile functions is expressed in a wide variety of mammalian tissues and cell types. It crosslinks intermediate filaments with microtubules and microfilaments and anchors them to junctional complexes [1]. Plectin is also up-regulated in various types of tumors and can serve as specific biomarker for pancreatic cancer [2]. However, the role of the plectin in hepatocellular carcinoma (HCC) metastasis and invasion is poorly understood.

To study the function of plectin in HCC, we established six different plectin knockout (KO) human HCC cell lines spanning the whole grading spectrum from well-differentiated to undifferentiated HCC [3]. These lines were characterized in wound healing migration, 3D spheroid invasion and anchorage-independent colony formation assays. In addition, we characterized impact of plectin deficiency on epithelial-mesenchymal transition. Altogether, we show in vitro the role of plectin in migration, invasion and tumorigenesis of HCC.

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[3] H. Yuzugullu et al., Mol. Cancer, vol. 8, no. 1, p. 90, Oct. 2009.

TbPH1: a kinetoplastid-specific pleckstrin homology domain containing kinesin-like protein

Hashimi H.
The Czech Academy of Sciences and University of South Bohemia, České Budějovice, Czech Republic

Trypanosoma brucei, the causative agent of a disease commonly known as sleeping sickness, a highly motile flagellate whose cytoskeleton has been a focus of much research. The kinesin and kinesin-like protein superfamily is among the largest in T. brucei. A member of this family is TbPH1, a protein containing a pleckstrin homology (PH) domain that inspired its name. This multidomain protein is made up of an N-terminal kinesin domain, whose Walker A motif is ablated by a single substitution, making its role as a microtubule motor suspect. TbPH1 also contains a coiled-coil region followed by PH and helix-turn-helix domains, suggesting interactions with other proteins and lipids. RNAi-silencing of TbPH1 in the two T. brucei life cycle stages compromises parasite fitness, likely due to a cell cycle defect resulting in an accumulation of G2-phase cells. In situ epitope-tagging reveals a punctate pattern throughout the cell, but often enriched between the nucleus and kinetoplast, the large mitochondrial DNA network that is the defining character of the order. Its localization predominantly excludes such organelles as the mitochondrion, endoplasmic reticulum and acidocalcisomes. Fractionation of T. brucei into cytoskeleton and soluble fractions does not support TbPH1 being a component of the former. However, TbPH1 is trapped by a technique called microtubule sieving, which takes advantage of the parasite's cage-like microtubule corset. This sieve lets cytosolic proteins diffuse away while capturing larger elements like stress-granules. The trypanosome ortholog of kinesin family member 11 (TbKIF11) co-immunoprecipitates with TbPH1 under the high salt conditions required to depolymerize the microtubules. In opisthokont (metazoan and fungi) model systems, KIF11 has been shown to play a key role in bipolar spindle assembly and elongation during mitosis. The observation that TbPH1 may interact with TbKIF11 suggests that they may work in concert in T. brucei, which undergoes closed mitosis.

Resolving controversy of unusually high refractive index of tubulin

Křivosudský O.
Institute of Photonics and Electronics, The Czech Academy of Sciences, Prague, Czech Republic

Refractive index of tubulin is an important parameter underlying fundamental electromagnetic and biophysical properties of microtubules - protein fibers essential for several cell functions including cell division. Yet, the only experimental data available in the current literature show values of tubulin refractive index (n = 2.36 - 2.90) which are much higher than established theories predict based on the weighted contribution of the polarizability of individual amino acids constituting the protein. To resolve this controversy, we report here modeling and rigorous experimental analysis of refractive index of purified tubulin dimer. Our experimental data revealed that the refractive index of tubulin is n = 1.64 at the wavelength 589 nm and 25 °C, that is much closer to the values predicted by established theories than the earlier experimental data provide.

Analysis of spatial segregation of the eIF3a mutant in Saccharomyces cerevisiae

Senohrábková L., Malcová I., Hašek J.
Institute of Microbiology, The Czech Academy of Sciences, Prague, Czech Republic

Yeast Saccharomyces cerevisiae has elaborated a strategy by which potentially harmful protein aggregates are retained within the mother cell and the pristine daughter cells have the full replicative potential. This process is called rejuvenation and it is a typical example of spatial protein quality control (SQC).

Here we present a mutant form of the translation initiation factor eIF3a/ Rpg1 - rpg1-3 that is an aggregate-prone protein since it forms foci already at physiological growth conditions. There two major types of rpg1-3 foci observable, small ones that are mobile and reach the daughter cell cytosol, and large ones that display a restricted mobility and are retained within the mother cell in the proximity of the nucleus. Ability of small rpg1-3 accumulations to escape from SQC of the mother cell is intriguing because it resembles a prion-like behavior, however rpg1-3 does not form amyloids. A microscopy analysis of the movement of small GFP-labeled rpg1-3 foci after application of 2-deoxyglucose and FCCP when the foci became immobile lead to a conclusion that their movement is active and dependent on energy. A trajectory analysis of the foci motility clearly showed that the foci movement is directed mostly from mother to daughter cell. It seems that intact microtubules are dispensable for the movement of rpg1-3 foci since their depolymerization by nocodazole affected neither the foci trajectory nor the velocity. However, depolymerization of actin cables by Latrunculin B interrupted the movement of foci to daughter cells. Surprisingly, we did not observed any changes in the heredity of rpg1-3 foci at the absence of the actin-based motor Myo4 and the protein adaptor She3. It seems thus that other transport molecules dependent on intact actin cables might carry rpg1-3 foci into daughter cells.

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Diverse subcellular localization of selected plant Class II formins

Šliková E, Stillerová L., Oulehlová D., Cvrčková F.
Department of Experimental Plant Biology, Faculty of Sciences, Charles University, Prague, Czech Republic

Formins are widely expressed family of proteins with a key role in the nucleation, binding and bundling of actin. Considering their biochemical properties, formins have various physiological functions which are often related to polarized growth, cell division, reorganization and spatial organization of actin and microtubules cytoskeleton. These large multidomain proteins are members of an evolutionarily conserved family, defined by the presence of conserved catalytic formin homology 2 (FH2) domain. To date 21 formin-encoding loci in two distinct subfamilies, Class I and Class II respectively, have been found in the model plant Arabidopsis thaliana. Both subfamilies share two conserved domains FH1 and FH2 on C terminus, whereas the N terminus of plant formins is distinct (Cvrčková et al., 2004).

While the roll of formins from Class I is studied more intensely, little attention is paid to the formins from Class II. We thus focused our attention on characterization of Arabidopsis formin AtFH13 (At5g58160) and AtFH14 (At1g31810) from Class II. To gain insights into the cellular sites of this closely related formins we studied their subcellular localization pattern after in vivo transient expression in N. benthamiana leaves or stabile expression in A. thaliana. We fused the AtFH13 and AtFH14 genes to YFP tag under the control of the UBQ promoter to facilitate their detection. Because microtubules (MT) localization is a hallmark of AtFH14 formin , we also transiently co-express the RFP-labeled MT markers and YFP-labeled AtFH13 and AtFH14 and we observed the association of these formins to the MT cytoskeleton which may indicate role in MT organizations and dynamics.

Membrane attachment of WASH complex

Humhalová T., Dostál V., Libusová L.
Faculty of Science, Charles University, Prague, Czech Republic

One of the neglected functions of the actin network is its role in endosomal protein sorting. Small patches of branched actin are assembled on endosomal membranes through the action of Arp2/3 complex and serve as microdomains into which the sorted membrane proteins are concentrated (1). However, Arp2/3 dependent nucleation of branched actin filaments needs to be promoted by a nucleation promoting factor. On early and sorting endosomes, this is achieved by pentameric WASH complex (2).

We are interested in WASH complex recruitment to the endosomal membrane. According to some reports, connection between WASH complex subunit FAM21 and retromer drives this recruitment. When this connection is lost, WASH delocalizes from endosomal membranes (3). In our hands, no such delocalization can be observed. In addition, WASH complex attachment to membranes is conserved in lower eukaryotes - unlike the interaction between WASH complex and retromer. Knockout cell line lacking another WASH complex subunit strumpellin revealed that this protein is not needed for the membrane recruitment.

Several WASH complex subunits delocalized upon SWIP knockout in Dictyostelium discoideum, opening the possibility that SWIP is responsible for the membrane attachment (4). We therefore expressed various fragments of SWIP in HeLa cells. Our preliminary data show that C-terminal part of SWIP is capable of localizing to membranes, although it does not interact with the rest of the complex. Since these observations are in line, SWIP remains our strongest candidate for WASH complex recruitment to the membrane.

(1) Puthenveedu et al. (2010), Cell. Nov 24;143(5):761-73
(2) Derivery et al. (2009), Dev Cell. Nov;17(5):712-23
(3) Harbour et al. (2010), J Cell Sci. Nov 1;123(Pt 21):3703-17
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Single-molecule study of cytoskeletal cross-linkers involved in cytokinesis

Kučera O.
BIOCEV, Institute of Biotechnology, The Czech Academy of Sciences, Prague, Czech Republic

Cell shape change and development rely on an interplay between actin and microtubule cytoskeletons. Although the crosstalk between these two networks is apparently mediated by a wide range of molecular cross-linkers and regulators, little is known about the interaction of these molecules with their cytoskeletal partners. Here we report on the biophysical characterization of the interaction of scaffolding proteins involved in cytokinesis with the cytoskeleton. Using optical trapping and fluorescence imaging, we directly observed the crucial role of these proteins in bundling and cross-linking of cytoskeletal elements reconstituted in vitro. Our results demonstrate the potential of the bottom-up approach to decipher complex networks of interactions, which can hardly be understood from experiments in cells.