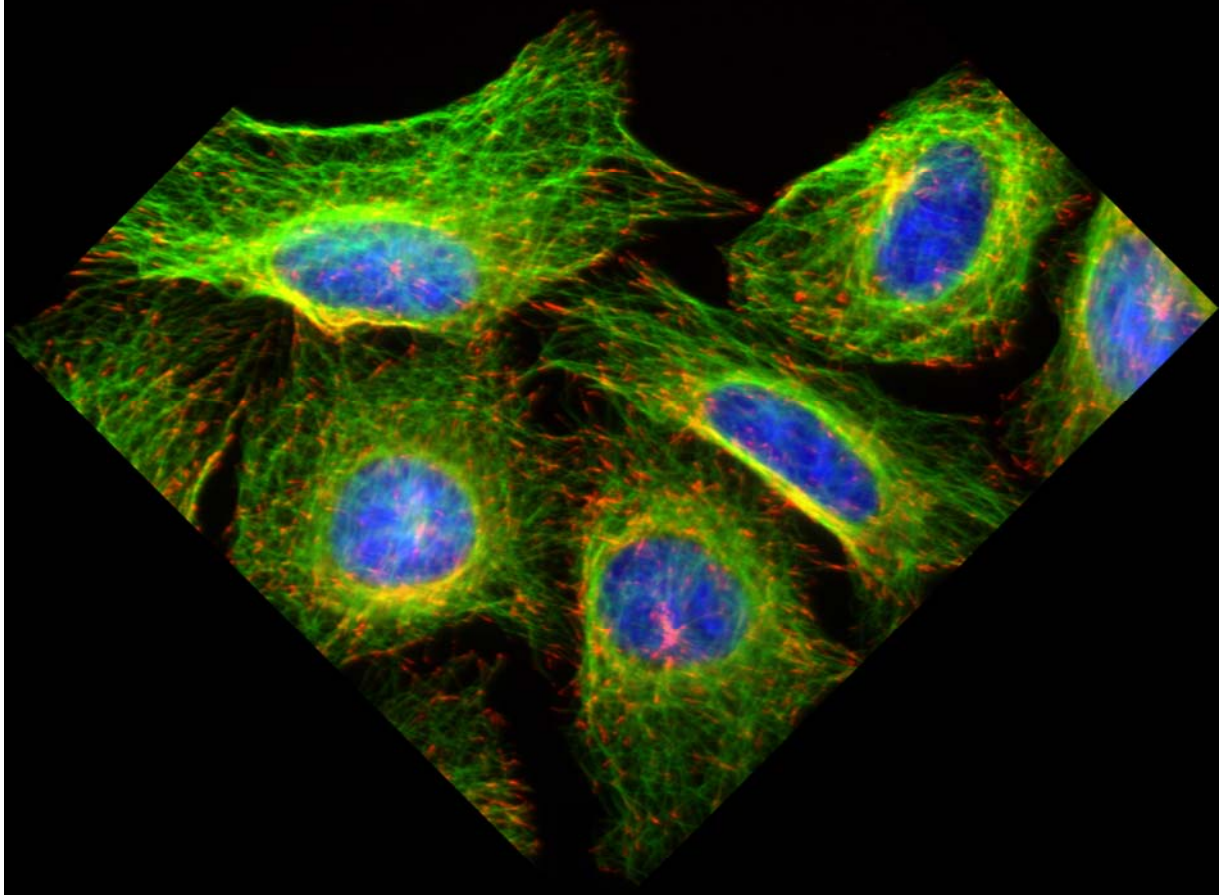
18TH CYTOSKELETAL CLUB



5th - 7th May 2010

Vranovská Ves

Institute of Molecular Genetics of the ASCR, v.v.i.

Czechoslovak Biological Society,
Section for Cell Biology

18TH CYTOSKELETAL CLUB

5th – 7th May 2010

Hotel Club, Vranovská Ves

Sponsors:

PROGRAMME

Wednesday, May 5, 2010

15:00 – 18:00 *Registration*

18:30 – 20:00 *Dinner*

20:00 – 21:00 **Urban Edit** (invited lecture)
Institute of Molecular Biotechnology, Vienna
ELECTRON TOMOGRAPHY REVEALS UNBRANCHED NETWORKS OF ACTIN FILAMENTS IN LAMELLIPODIA

21:00 *Discussion & wine tasting*

Thursday, May 6, 2010

7:30 – 9:00 *Breakfast*

9:00 – 9:45 **Widlund O. Per** (invited lecture)
Max Planck Institute of Molecular Cell Biology and Genetics, Dresden
XMAP215 REQUIRES TOG DOMAINS AND A TETHER TO THE MICROTUBULE FOR POLYMERIZATION

9:45 – 10:30 **Varga Vladimir** (invited lecture)
Max Planck Institute of Molecular Cell Biology and Genetics, Dresden
CHARACTERIZATION OF *SACCHAROMYCES CEREVISIAE* KINESIN-8 BY SINGLE-MOLECULE FLUORESCENCE MICROSCOPY

10:30 – 10:50 *Coffee break*

10:50 – 11:35 **Dráber Petr** (invited lecture)
Institute of Molecular Genetics AS CR, Prague
ACTIN CYTOSKELETON IN IMMUNORECEPTOR SIGNALING

11:35 – 12:00 **Šolc Petr**
Institute of Animal Physiology and Genetics AS CR, Libechov
AURORA-A REGULATES MTOCs (CENTROSOME) BIOGENESIS BUT IT DOES NOT TRIGGER G2/M TRANSITION IN MOUSE OOCYTES MATURATED *IN VIVO*

12:00 – 12:25 **Cenklová Věra**
Institute of Experimental Botany AS CR, Olomouc
THE ROLE OF At AURORA1 KINASE IN ACENTROSOMAL PLANT CELLS

12:25 – 12:40 **Sulimenko Vadym**
Institute of Molecular Genetics AS CR, Prague
RECOVERY OF TUBULIN FUNCTIONS AFTER FREEZE-DRYING IN THE PRESENCE OF TREHALOSE

12:45 – 14:15 *Lunch*

14:30 – 15:00 Meeting of the Center for Study of Functional Organization of the Cell

15:00 – 15:25 **Binarová Pavla**
Institute of Microbiology AS CR, Prague
ARABIDPOSIS NodG IS A REGULATORY PROTEIN ASSOCIATED WITH MICROTUBULAR CYTOSKELETON

15:25 – 15:45 **Fendrych Matyáš**
Institute of Experimental Botany AS CR, Prague
PLANT FORMIN AtFH4 INTERACTS WITH MICROTUBULE CYTOSKELETON

15:45 – 16:10 **Pleskot Roman**
Institute of Experimental Botany, AS CR, Prague
PHOSPHATIDIC ACID: ITS ROLE IN THE REGULATION OF ACTIN DYNAMICS

16:10 – 16:30 **OLYMPUS** presentation

16:30 – 16:50 *Coffee break*

16:50 – 17:15 **Kobrlová Jana**
Department of Plant Physiology, Charles University, Prague
HSP90 IS A MICROTUBULE-INTERACTING PROTEIN

17:15 – 17:30 **Čížková Mária**
Institute of Microbiology AS CR, Třeboň
DNA DAMAGE CHECKPOINT IN GREEN ALGA *CHLAMYDOMONAS REINHARDTII*

17:30 - 17:45 **Hlavová Monika**
Institute of Microbiology AS CR, Třeboň
IDENTIFICATION OF THERMOSENSITIVE CDC MUTANTS IN *CHLAMYDOMONAS REINHARDTII*

17:45 – 18:00 **ROCHE** presentation

18:00 – 19:00 Meeting of the Committee of the Society for Cell Biology of CSBS

19:00 *Raut & wine tasting*

Friday, May 7, 2010

7:30 – 9:00	*Breakfast*
9:30 – 9:55	**Dráber Pavel** *Institute of Molecular Genetics AS CR, Prague* ESSENTIAL ROLE OF STIM1 IN MICROTUBULE REORGANIZATION DURING ACTIVATION OF MAST CELLS
9:55 – 10:20	**Vinopal Stanislav** *Institute of Molecular Genetics AS CR, Prague* IDENTIFICATION OF A NOVEL GAMMA-TUBULIN-ASSOCIATED PROTEIN
10:20 – 10:35	**Holubcová Zuzana** *Faculty of Medicine, Masaryk University, Brno* TO DIVIDE OR NOT TO DIVIDE? HOW HUMAN EMBRYONIC STEM CELLS DEAL WITH EXTRA CENTROSOMES?
10:35 – 10:55	*Coffee break*
10:55 – 11:20	**Groušl Tomáš** *Institute of Microbiology AS CR, Prague* HEAT SHOCK AT 42ºC RESULTS IN ACCUMULATION OF DCP2 AND NGR1 PROTEINS INDEPENDENTLY OF P-BODY SCAFFOLDS
11:20 – 11:35	**Hašek Jiří** *Institute of Microbiology AS CR, Prague* CYTOSKELETON AND HEAT-INDUCED STRESS GRANULES IN *S. CEREVISIAE*
11:35 – 11:50	**Sůkal Petr** *Faculty of Mechanical Engineering, University of Technology, Brno* COMPUTATIONAL MODELLING OF MECHANICAL TESTS OF CELLS
11:50 – 12:00	Concluding remarks
12:00	*Lunch*

ELECTRON TOMOGRAPHY REVEALS UNBRANCHED NETWORKS OF ACTIN FILAMENTS IN LAMELLIPODIA

Edit URBAN*[1], Sonja JACOB*[1], Maria NEMETHOVA[1], Guenter P. RESCH[1,2], J. V. SMALL[1]

[1]*Institute of Molecular Biotechnology, Vienna, Austria*
[2]*Research Institute of Molecular Pathology, Vienna, Austria*
**Equal contribution*

Cells initiate movement using the forces exerted by polymerizing actin filaments to extend lamellipodia and filopodia protrusions. In a current model, actin filaments in lamellipodia are organized in a branched, dendritic network. We have now applied electron tomography to vitreously frozen "live" cells, fixed cells and cytoskeletons, embedded in vitreous ice or in deep negative stain. In lamellipodia from four cell types, including rapidly migrating fish keratocytes, we found that actin filaments are almost exclusively unbranched: the vast majority of apparent filament junctions proved to be overlapping filaments and branched end-to-side junctions were rare. Analysis of the tomograms revealed that actin filaments terminate at the membrane interface within a zone several hundred nm wide at the lamellipodium front and yielded the first direct measurements of filament densities. Actin filament pairs were also identified as lamellipodium components and bundle precursors. The data provide a new structural basis for understanding actin-driven protrusion during cell migration.

XMAP215 REQUIRES TOG DOMAINS AND A TETHER TO THE MICROTUBULE FOR POLYMERIZATION

Per O. WIDLUND[1,4], Jeffrey H. STEAR[2,4], Simone REBER[1], Andrei POZNIAKOVSKY[1], Gary J. BROUHARD[3] , Jonathon HOWARD[1], Anthony A. HYMAN[1]

[1]*Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany*
[2]*Max Delbrük Center for Molecular Medicine, Berlin-Buch, Germany*
[3]*Department of Biology, McGill University, Montréal, Québec, Canada*
[4]*These authors contributed equally to this work*

During cell division, different proteins adjust the dynamic properties of microtubules so that they can be used to carry out various cellular functions such as chromosome segregation, vesicle transport, and cell motility. The XMAP215/Dis1 family of proteins has been shown to dramatically promote microtubule growth and its activity can account for most of the rapid growth rates of microtubules seen in cells. Recent work has shown that the *Xenopus* member of this family, XMAP215, acts as a catalyst, stabilizing the transition state of a tubulin subunit while it is incorporated into the microtubule polymer. For this reason, it has been called a polymerase. XMAP215/Dis1 family proteins are characterized by N-terminal repeats called TOG domains. TOG domains bind tubulin and are thought to be central to their activity, but the mechanism by which the TOG domains accelerate microtubule growth rate is not known. We dissected the roles of the different TOG domains in XMAP215 and show that mutation of individual and combinations of TOG domains have strikingly different effects on polymerization activity. TOGs 1 and 2 are critical for activity while TOGs 3, 4, and 5 contribute minimally. However, a TOG12 fragment is not sufficient because a high affinity microtubule-binding domain is required for TOGs to be targeted to ends where they can polymerize microtubules. Our results suggest that multiple TOG domains increase the affinity for tubulin dimers resulting in a higher maximal growth rate while a high affinity microtubule lattice-binding domain is important for efficient function at low concentrations of XMAP215.

CHARACTERIZATION OF *SACCHAROMYCES CEREVISIAE* KINESIN-8 BY SINGLE-MOLECULE FLUORESCENCE MICROSCOPY

Vladimir VARGA, Cecile LEDUC, Stefan DIEZ, Jonathon HOWARD

Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany

Kinesins are a large superfamily of eukaryotic proteins, which convert chemical energy of ATP hydrolysis into mechanical work. Most kinesins walk along microtubules, thereby translocating cargo such as vesicles and organelles in the cell. However, members of the kinesin-13 family do not translocate but instead destabilize microtubules by depolymerizing them from their ends. Using total-internal-reflection-fluorescence microscopy and purified proteins we discovered that the *Saccharomyces cerevisiae* kinesin-8, Kip3p, combines these two activities. It is a highly processive plus-end-directed motor and it is also a microtubule depolymerase. As a consequence of these single-molecule properties, the rate of depolymerization of GMP-CPP-stabilized microtubules by ensemble of Kip3p is length-dependent. This could provide a new mechanism for the cell to control the length of microtubule-based structures such as the mitotic spindle.

When depolymerization of microtubules is blocked by stabilizing them with GMP-CPP and taxol, the dissociation rate of Kip3p from the plus end is rather low ($< 0.1\ s^{-1}$). These end-bound molecules form an obstacle, which causes accumulation of incoming motors leading to formation of a high motor-density region proximal to the plus end. The abrupt increase in the motor density correlates with the abrupt decrease in their velocity, the phenomenon thus being similar to traffic jams on highways. This in vitro system helps us to understand how motors behave on crowded tracks in the cellular environment.

ACTIN CYTOSKELETON IN IMMUNORECEPTOR SIGNALING

Petr DRÁBER

Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Prague, Czech Republic

The earliest known biochemical step that occurs after ligand binding to the multichain immune recognition receptor is tyrosine phosphorylation of the receptor subunits. In mast cells and basophils activated by multivalent antigen-IgE complexes this step is mediated by Src family kinase Lyn, which phosphorylates the high affinity IgE receptor (FcεRI). However, the exact molecular mechanism of this phosphorylation step is incompletely understood. In our laboratory we study the role of actin cytoskeleton in the earliest stages of mast cell signaling using mouse bone-marrow-derived mast cells (BMMCs). We have found that cells deficient in the transmembrane adaptor protein NTAL exhibit changes in cell morphology and higher calcium and secretory responses than wild-type (WT) cells [1, 2]. In this presentation I will show new data indicating that BMMCs from $NTAL^{-/-}$ mice, responding to Ag alone or in combination with stem cell factor (SCF), exhibit reduced spreading to the connective tissue component, fibronectin, and enhanced filamentous actin depolymerization relative to WT cells. No such difference was observed when SCF alone was used as activator. We have examined activities of two small GTPases, Rac and Rho, important regulators of actin polymerization. Stimulation with antigen and/or SCF enhanced activity of the Rac(1,2,3) in both $NTAL^{-/-}$ and WT cells. In contrast, the RhoA activity decreased and this trend was much faster and more extensive in $NTAL^{-/-}$ cells, indicating a positive regulatory role of NTAL in the recovery of RhoA activity. After restoring NTAL into $NTAL^{-/-}$ cells, both spreading and actin responses were rescued. We also observed changes in antigen-induced chemotaxis between WT and $NTAL^{-/-}$ BMMC, suggesting involvement of NTAL in cell movement.

Cytoskeleton seems to be also involved in the first signaling step, leading to tyrosine phosphorylation of the receptor. We found that exposure of BMMCs to protein tyrosine phosphatase (PTP) inhibitors, H_2O_2 or pervanadate, induced phosphorylation of the FcεRI subunits, similarly as FcεRI triggering. Interestingly, and in sharp contrast to antigen-induced activation, neither H_2O_2 nor pervanadate induced any changes in the association of FcεRI with detergent-resistant membranes and in the topography of FcεRI detectable by electron microscopy on isolated plasma membrane sheets. In cells stimulated with pervanadate, H_2O_2 or antigen enhanced oxidation of active site cysteine of several PTPs was detected. Unexpectedly, most of oxidized phosphatases bound to the plasma membrane were associated with actin cytoskeleton. Based on these and other data we propose that down-regulation of enzymatic activity of PTPs and/or changes in their accessibility to the substrates play a key role in initial tyrosine phosphorylation of the FcεRI and other multichain immune receptors. Actin cytoskeleton could play a critical role in this process.

1 Volná, P. et al., Negative regulation of mast cell signaling and function by the adaptor LAB/NTAL. *J. Exp. Med.* 2004. 200: 1001-1013.

2 Dráberová, L. et al., Regulation of Ca^{2+} signaling in mast cells by tyrosine-phosphorylated and unphosphorylated non-T cell activation linker. *J. Immunol.* 2007. 179: 5169-5180.

AURORA-A REGULATES MTOCs (CENTROSOME) BIOGENESIS BUT IT DOES NOT TRIGGER G2/M TRANSITION IN MOUSE OOCYTES MATURATED *IN VIVO*

Petr ŠOLC[1], Vladimir BARAM[2], Gabriela PANENKOVÁ[1], Adéla ŠAŠKOVÁ[1], Richard M. SCHULTZ[3] and Jan MOTLÍK[1]

[1] *Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, Libechov, Czech Republic*
[2] *Institute of Animal Physiology, Slovak Academy of Sciences, Kosice, Slovakia*
[3] *Department of Biology, University of Pennsylvania, Philadelphia, USA;*

Aurora-A kinase (AURKA) is an important mitotic kinase involved in G2/M transition, centrosome separation and spindle formation in somatic cells. Almost completely all known information about a role of AURKA in cell cycle regulation is derived from in vitro cultured human cancer cells. Using transgenic models we provide insight on the physiological role of AURKA during meiotic maturation (meiotic cell cycle progression) in mice. By means of Cre-lox system we have generated mice with expression of both wild-type (wt) and kinase-dead (kd) aurka transgenes specifically only in ovarian oocytes. Intrafollicularly, oocytes are arrested at prophase I and they resume meiosis in vivo (G2/M transition) after LH/hCG stimuli. AURKA activation occurs shortly after LH/hCG peak. Although expression of wt aurka transgene leads to premature activation of AURKA at prophase I oocytes they do not resume meiosis without LH/hCG stimuli. The control oocytes contain 1-3 microtubule organizing centres (MTOCs; centrosome equivalent) at prophase I. At the time of nuclear envelope break down (NEBD), a first visible marker of meiosis resumption, MTOCs number increases up to several dozen (MTOCs multiplication). After wt aurka transgene expression in mouse oocytes, MTOCs multiplication occurs prematurly at prophase I without NEBD although both control and kd aurka mice exhibit usually 1-3 MTOCs per oocytes. At metaphase I stage wt aurka oocytes have longer spindle than control and kd aurka oocytes. Both wt aurka nad kd aurka transgenic mice have normal fertility during first 7 mouth of their life. It suggests that the AURKA induced premature MTOCs multiplication does not interfere with developmental competence of oocytes. In conclusion these data imply that AURKA activity is responsible for physiological MTOCs multiplication and for regulation of the spindle length but that AURKA is not a trigger kinase for G2/M transition in mouse oocytes.

THE ROLE OF At AURORA1 KINASE IN ACENTROSOMAL PLANT CELLS

Věra CENKLOVÁ[1], Beáta PETROVSKÁ[1], Žaneta POCHYLOVÁ[1], Zuzana GELOVÁ[1], Lenka BINAROVÁ[1], Anna DOSKOČILOVÁ[2], Pavla BINAROVÁ[2]

[1]*Institute of Experimental Botany, Academy of Sciences of the Czech Republic, Olomouc, Czech Republic*
[2]*Institute of Microbiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic*

Three Aurora kinase genes (At Aurora1, At Aurora2 and At Aurora3) presented in the genome of *Arabidopsis thaliana* are phylogenetically distant from mammalian A, B, and C Aurora kinases. To get better insight into the function of plant Aurora kinases we decided to characterize At Aurora1. Cellular localization of At Aurora1 and its activator At TPX2 during cell cycle progression were analyzed in *Arabidopsis* plants and in cultured cells. At Aurora1 kinase and its activator At TPX2 protein localized with the pre-prophase band of cortical microtubules, associated with the prophase and the metaphase mitotic spindle, localized along the entire length of kinetochore fibres of the metaphase spindle and with shortening anaphase kinetochore fibres on the poles. On mitotic exit active At Aurora1/At TPX2 protein moved from the poles to a specific subset of early phragmoplast microtubules in the vicinity of chromatin. During telophase, the At Aurora1 concentrated at the forming cell plate where persisted until end of cytokinesis while At TPX2 was degraded. The RNAi expressing plants with reduced kinase levels as well as and T-DNA insertion mutants showed similar phenotype with severe cell division and cytokinetic defects, affected meristems development, defects of polar growth and cell patterning.

Our data showed that At Aurora1 has conserved functions in the regulation of multiple steps of cell division, in organization of acentrosomal microtubular arrays and thus has properties of Aurora A and Aurora B type of kinase of vertebrates.

Supported by grants GACR 204/07/1169, MSMT LC06034, GACR 204/09/P155, LC 545, and GA AVCR 500200719.

RECOVERY OF TUBULIN FUNCTIONS AFTER FREEZE-DRYING IN THE PRESENCE OF TREHALOSE

Vadym SULIMENKO[1], Eduarda DRÁBEROVÁ[1], Tetyana SULIMENKO[1], Konrad J. BŐHM[2], Pavel DRÁBER[1]

[1]*Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Prague, Czech Republic*
[2] *Leibniz Institute for Age Research- Fritz Lipmann Institute, Jena, Germany*

Microtubules represent cytoplasmic structures that are indispensable for the maintenance of cell morphology and motility generation. Due to their regular structural organization microtubules have become of great interest for preparation of *in vitro* nanotransport systems. However, tubulin, the major building protein of microtubules, is a thermolabile protein and is usually stored at -80 °C to preserve its conformation and polymerization properties. Here we describe a novel method for freeze-drying of assembly-competent tubulin in the presence of a non-reducing sugar trehalose. Even after prolonged storage at ambient temperature, rehydrated tubulin is capable of binding anti-mitotic drugs and assembling to microtubules that bind microtubule-associated proteins in usual way. Electron microscopy confirmed that rehydrated tubulin assembles into normal microtubules which are able to generate motility by interaction with the motor protein kinesin in cell-free environment. Freeze-drying also preserved pre-formed microtubules. Rehydrated tubulin and microtubules can be used for preparation of diverse *in vitro* and *in vivo* assays as well as for preparation of bionanodevices.

This work was supported by the grant KAN200520701 from GA ASCR and grant LC545 from Ministry of Education, Youth and Sports of the Czech Republic.

ARABIDPOSIS NodG IS A REGULATORY PROTEIN ASSOCIATED WITH MICROTUBULAR CYTOSKELETON

Ondřej PLÍHAL, Anna DOSKOČILOVÁ, Jindřich VOLC, Petr HALADA, Jana CHLUMOVÁ, Pavla BINAROVÁ

Institute of Microbiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic

NodG presents yet uncharacterized Arabidopsis protein that was identified in our proteomic studies among proteins interacting with plant microtubules and with gamma-tubulin. Although NodG homologues are present in many sequenced plant genomes function of the protein is not yet understood. NodG is fusion proteins composed of N-terminal part with amidohydrolase domain that shares homology with several nodulins involved in early or late response of leguminous plant to bacterial root infection, and C-terminal part homologous to prokaryotic glutamine synthetase type I (GSI) that is a central component of nitrogen metabolism. NodG complexes of size corresponding to octamer were found in cytoplasm, with membranes and microtubules, as well as co-purified with gamma-tubulin. NodG exhibited glutamine synthetase activity; accordingly, phenotype of NodG RNAi plants with inhibited early root growth and enhanced branching of lateral roots resembled root phenotype induced by addition of exogenous glutamate. Further, NodG expression was found to be highly responsive to bacterial flagellin treatment. Altogether our data showed that NodG fusion protein had GS activity comparable with prokaryotic GSI. Changes in root development observed in NodG depleted RNAi plants might thus reflect impaired regulatory function of NodG in Glu/Gln related signalling pathways. We found further that expression of NodG was triggered by elicitor treatment and presence of nodulin like part of molecule suggest that analogy to bacterial elicitation of the family of nodulins might exist for NodG fusion protein. Association with microtubular cytoskeleton might provide flexible platform for NodG as a regulatory protein in morphogenic processes related to Glu/Gln signalling and pathogen elicitation

Supported by The Grant Agency of the ASCR grants KJB500200705 and IAA500200719 and MSMT LC545

PLANT FORMIN AtFH4 INTERACTS WITH MICROTUBULE CYTOSKELETON

Matyáš FENDRYCH[1,2], Michael J. DEEKS[3], Fatima CVRČKOVÁ[2], Patrick J. HUSSEY[3], Viktor ŽÁRSKÝ[1,2]

[1]*Faculty of Sciences, Charles University, Prague, Czech Republic*
[2]*Institute of Experimental Botany, Academy of Sciences of the Czech Republic, Prague, Czech Republic*
[3]*School of Biological and Biomedical Sciences, University of Durham, Durham, United Kingdom*

Formins form a large family of eukaryotic proteins sharing the evolutionarily conserved FH2 (formin homology 2) domain that has been shown to nucleate actin. Formin dimers act as a processive or "leaky" cap at the barbed ends or associate with the side of actin filaments and participate in forming actin bundles.

Arabidopsis thaliana genome encodes over 20 formin proteins, all of which contain plant-specific regions in place of the GTPase-binding domain, formin homology (FH)3 domain, and DAD and DID motifs found in many fungal and animal formins. Here we show that Arabidopsis formin AtFH4 associates with microtubule cytoskeleton in vivo. We have identified a plant - specific domain of this protein that mediates microtubule binding. In vitro analysis shows that this region binds directly to microtubules.

Expression of AtFH4 caused endoplasmatic reticulum co-alignment with microtubules, showing that this protein is able to simultaneously associate with microtubules and the membrane. Recombinant AtFH4 was also able to nucleate actin in vitro, we have however not proven actin-microtubule coaligment in vivo.

We propose that AtFH4 might integrate membranes, microtubular and actin cytoskeletal networks.

PHOSPHATIDIC ACID: ITS ROLE IN THE REGULATION OF ACTIN DYNAMICS

Roman PLESKOT[1], Martin POTOCKÝ[1], Přemysl PEJCHAR[1], Jan LINEK[2], Radek BEZVODA[3], Jan MARTINEC[1], Olga VALENTOVÁ[2], Zuzana NOVOTNÁ[2], Chris J. STAIGER[4] and Viktor ŽÁRSKÝ[1,3]

[1]*Institute of Experimental Botany, Academy of Sciences of the Czech Republic,Prague , Czech Republic*

[2]*Institute of Chemical Technology, Faculty of Food and Biochemical Technology, Prague, Czech Republic*

[3]*Charles University, Faculty of Science, Prague, Czech Republic*

[4]*Purdue University, Department of Biological Sciences, West Lafayette, USA*

Membrane lipids and cytoskeleton dynamics are intimately inter-connected in the eukaryotic cell; however, only recently the molecular mechanisms operating at this interface in plant cells have been addressed experimentally. Phosphatidic acid (PA) was discovered to be an important regulator in the membrane–cytoskeleton interface in eukaryotes. PA can be produced via two independent pathways: either by phosphorylation of diacylglycerol (DAG) with DAG kinase, or by phospholipase D (PLD)-mediated hydrolysis of structural phospholipids

Here we report the mechanistic details of plant PLD–actin interactions. Inhibition of PLD by n-butanol compromises pollen tube actin, and PA rescues the detrimental effect of n-butanol on F-actin, showing clearly the importance of the PLD–PA interaction for pollen

tube F-actin dynamics. From various candidate tobacco PLDs isoforms, we identified NtPLDβ1 as a regulatory partner of actin, by both activity and *in vitro* interaction assays. We then described the NtPLDβ1 domain responsible for actin interactions. Using sequence- and structure-based analysis, together with site-directed mutagenesis, we identified crucial amino acids in the actin-interacting fold. The effect of antisense-mediated suppression of NtPLDβ1 or NtPLDδ on pollen tube F-actin dynamics shows that NtPLDβ1 is the active partner in PLD–actin interplay. The positive feedback loop created by activation of PLDβ by F-actin and of F-actin by PA provides an important mechanism to locally increase membrane–F-actin dynamics in the cortex of plant cells.

An important aspect of this positive feedback loop is a direct interaction of PA with actin-capping protein (CP). In our work we also described a putative PA-binding domain of CP using *in silico* approach. We constructed a model of 3D structure of CP from *Arabidopsis thaliana* by homology modeling. Comparison of our model with known structure from *Gallus gallus* (rcsb pdb: 1izn) shows differences in the distribution of electrostatic potential between these two counterparts and indicates putative binding site. To further identify the PA-binding domain we utilized a molecular docking strategy.

HSP90 IS A MICROTUBULE-INTERACTING PROTEIN

Jana KOBRLOVÁ[1], Aleksandra JOVANOVIĆ[2], Kateřina SCHWARZEROVÁ[1], Peter NICK[2]

[1]*Department of Plant Physiology, Charles University, Prague, Czech Republic*
[2]*Institute of Botany I, University of Karlsruhe, Karlsruhe, Germany*

Microtubules (MTs) play an important role in many cellular functions, mainly in plant cell shape control, plant development and cell division. Multitude of MT-associated proteins and interacting partners were described that influence microtubular dynamics, stability, and are involved in processes dependent on correct microtubular function such as signaling or cell growth. Using several methods we have shown that HSP90 is a microtubule-interacting protein, and some experiments implied that HSP90 interacts also with the plasma membrane. Further investigation revealed that HSP90 colocalised with cortical MTs in membrane ghosts obtained from tobacco cell lines stably expressing GFP-NtHSP90 or GFP-OsHSP82. HSP90 was also immunolocalised in phragmoplast, spindle and preprophase band in tobacco cells.

For in vitro analysis of HSP90 MT-binding function, we cloned the tobacco full-length cDNA sequence into the overexpression vector, expressed HSP90 in BL21(DE3).RIL cells and the recombinant protein was purified using Ni-NTA column. In cosedimentation analysis with polymerized tubulin HSP90 bound to microtubules, which demonstrated the direct interaction of HSP90 with MTs.

To investigate microtubule-binding HSP90 function in plant cells, HSP90 inhibitor geldanamycin (GDA) was used in further experiments. Whereas GDA had no effect on microtubular organization under control conditions, it suppressed the repolymerization of MTs after cold treatment.

Our results showed that HSP90 is the true microtubule-binding protein and that the inhibition of HSP90 resulted in changes of MTs reorganization in tobacco cells. Great diversity in morphological traits caused by GDA during stress in Arabidopsis plants reported by Queitsch et al. (2002) and Sangster and Queitsch (2005) could be thus partly explained by the inhibition of MTs that represent the main morphogenetic tool in plant cells.

This work was supported by the grant of the Ministry of Education of the Czech Republic ME10111, the DAAD project no. D14-CZ 14/2008-09, the Research program of the Ministry of Education of the Czech Republic LC06034 and the Research program of the Ministry of Education of the Czech Republic MSM 0021620858

References:

Queitsch, C.; Sangster, T.A. and Lindquist, S. (2002): Nature 417: 618-624
Sangster, T.A. and Queitsch, C. (2005): Current Opinion in Plant Biology 8: 86–92

DNA DAMAGE CHECKPOINT IN GREEN ALGA *CHLAMYDOMONAS REINHARDTII*

Mária ČÍŽKOVÁ[1], Monika HLAVOVÁ[1], Milada VÍTOVÁ[1], James G. UMEN[2], Kateřina BIŠOVÁ, Vilém ZACHLEDER[1]

[1]Institute of Microbiology, Academy of Sciences of the Czech Republic, Třeboň, Czech Republic
[2]*The Salk Institute for Biological Studies, La Jolla, USA*

Although cellular genome is relatively stable, it can be altered both spontaneously and after exposition to various DNA damaging agents. Cells usually respond to the genome damage by activating a DNA damage response pathway. In plants, DNA damage checkpoint is controlled by WEE1 kinase operating in ATM/ATR dependent manner. We studied the effects of zeocin, a bleomycin family antibiotics causing double stranded breaks, and caffeine, methylxantine synergistic with many DNA damaging agents, on the growth rate, cell survival and cell cycle progression of the alga *Chlamydomonas reinhardtii*. Increasing concentrations of zeocin severely affected cell survival and blocked nuclear and cell divisions. *Chlamydomonas*‘ cells were hypersensitive to DNA damage if combined with the presence of caffeine. In the presence of caffeine only, the cells divided faster comparing to untreated control, proably due to degradation of inhibitor of mitosis, WEE1. Moreover, we analyzed expression and activity of CDKB1 and WEE1 kinases in response to DNA damage. Both genes were transcriptionally induced upon DNA damage and post-transcriptionally regulated in ATM/ATR dependent manner, leading to a cell cycle arrest prior to S/M phase with inactive CDKB1. Here, we show that application of caffeine can override a cell cycle arrest following to DNA damage. We conclude that CDKB1 is major mitotic kinase in *C. reinhardtii* and it is also major target of WEE1 kinase. Both the proteins are required for the response to DNA damage.

This work was supported by the GA CR (grant nos. 204/09/0111, P501/10/P258), the GA AS CR (grant no. IAA500200614) and the Institutional Research Concept (no. AV0Z50200510).

IDENTIFICATION OF THERMOSENSITIVE CDC MUTANTS IN *CHLAMYDOMONAS REINHARDTII*

Monika HLAVOVÁ, Mária ČÍŽKOVÁ, Vilém ZACHLEDER, Kateřina BIŠOVÁ

Institute of Microbiology, Academy of Sciences of the Czech Republic, Třeboň, Czech Republic

Because cell division cycle (cdc) genes encode essential proteins, a genetic approach to dissect the molecular mechanisms of the cell cycle has relied on the isolation of conditional mutants. In most cases these are temperature sensitive mutants, which can grow at a lower (permissive) temperature but arrest at some point in the cell cycle at a higher (restrictive) temperature. The power of this approach is that mutations can be induced in unknown proteins and then studied.

The haploid biflagellate unicellular photosynthetic alga Chlamydomonas reinhardtii has many features, which make it ideal for the isolation of cell cycle mutants. Chlamydomonas divides by multiple fission, which suggest that Chlamydomonas will provide unique insight into the control and coordination of the cell cycle.

In our studies we used a chemical mutagenesis for the isolation of conditional cell cycle mutants. The ethyl-methanesulphonate (EMS) was used as a mutagen. It is a mild mutagen, which causes a high proportion of base pair substitutions in DNA. Obtained mutants were able to grow at 24°C (permissive temperature) but couldn't grow at 36 °C (restrictive temperature). In future the course of the cell cycle of these conditional mutants will be studied and molecular and genetic analysis will be performed.

This work was supported by the GA CR (grant no. 204/09/0111), the GA AS CR (grant no. IAA500200614) and the Institutional Research Concept (no. AV0Z50200510).

ESSENTIAL ROLE OF STIM1 IN MICROTUBULE REORGANIZATION DURING ACTIVATION OF MAST CELLS

Pavel DRÁBER, Zuzana HÁJKOVÁ, Viktor BUGAJEV, Eduarda DRÁBEROVÁ, Stanislav VINOPAL, Lubica DRÁBEROVÁ, Petr DRÁBER

Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Prague, Czech Republic

Activation of mast cells by aggregation of the high-affinity IgE receptors (FcεRI) initiates signaling events leading to the release of inflammatory and allergic mediators from cytoplasmic granules. This process, termed degranulation, involves regulated changes in the concentration of Ca^{2+} in the cytoplasm. Store operated Ca^{2+} entry (SOCE) plays a key role in this regulation. Although microtubules are important for degranulation, the molecular mechanisms that control changes in microtubule organization during mast cell activation are largely unknown.

Here we report that activation of mouse bone marrow-derived mast cells (BMMCs) induced by FcεRI aggregation, pervanadate or thapsigargin treatment leads to generation of microtubule protrusions in later stages of activation, characterized by Ca^{2+} influx. Changes in cytosolic Ca^{2+}concentration affected microtubule plus-end dynamics tracked by distribution of microtubule-plus-end tracking protein EB1. Knock-down of STIM1 (stromal interaction molecule 1), the principal regulator of SOCE, revealed that rearrangement of microtubules depends on the activity of STIM1. This finding was further corroborated by a rescue experiments with mutated STIM1. While relocation of STIM1 to the close proximity of the plasma membrane was microtubule-independent, STIM1 formed puncta associated with microtubule protrusions at later stages of activation. We propose that microtubules are modulated by SOCE and that Ca^{2+} plays an important role in signaling to microtubules in BMMCs.

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IDENTIFICATION OF A NOVEL GAMMA-TUBULIN- ASSOCIATED PROTEIN

Stanislav VINOPAL, Věra RICHTEROVÁ, Eduarda DRÁBEROVÁ, Pavel DRÁBER

Institute of Molecular Genetics, Academy of Sciences of the Czech Republic, Prague, Czech Republic

Gamma-tubulin is the key player in microtubule nucleation and organization. However, new findings have been emerging about its role in other cellular processes including spindle assembly checkpoint, establishment of spindle bipolarity by interactions with mitotic kinesins, pre-anaphase spindle positioning in the yeast or regulation of MT (+) end dynamics. To find novel interaction partners of gamma-tubulin we performed immunoprecipitations with specific monoclonal and polyclonal anti-peptide antibody against gamma-tubulin from mouse embryonal carcinoma cell lysates and analyzed their composition by MALDI-TOF/MS. This analysis led to identification of a 90 kDa protein (K0776). This protein was very recently characterized as a regulator of C53 protein. The multifunctional C53 acts as a tumor suppressor as well as a centrosomal regulator of G2/M progression.

We have prepared EGFP-K0776 construct and established stable U2OS cell line. K0776 was found in the cytoplasm, on endoplasmic reticulum, and in a subpopulation of cells also on the centrosomes of living cells. Time-lapse imaging of the stable cell line revealed that EGFP-KIAA forms "rods" in the nucleus, which seem to be invaginations of endoplasmic reticulum into the nuclear space. The interaction among gamma-tubulin, K0776 and C53 was confirmed by immunoprecipitation experiments with polyclonal and monoclonal anti-gamma-tubulin antibodies or anti-GFP antibody using extracts from the EGFP-K0776 cell line. Moreover, interaction of gamma-tubulin with K0776 was confirmed in different cell types of various origins. Pull-down experiments with GST-tagged full-length gamma-tubulin and GST-tagged N- or C-terminal domains of K0776 from U2OS lysates revealed, that the N-terminal domain of K0776 is important for interaction with both gamma-tubulin and C53. Our studies indicate that novel gamma-tubulin complexes might play an important role in the regulation of cell cycle progression.

This work was supported by grant No. 204/09/H084 from GACR and grant No. KAN200520701 from GA ASCR.

TO DIVIDE OR NOT TO DIVIDE? HOW HUMAN EMBRYONIC STEM CELLS DEAL WITH EXTRA CENTROSOMES?

Zuzana HOLUBCOVÁ[1], Pavel MATULA[2], Miroslava SEDLAČKOVÁ[3], Tomáš BÁRTA[4], Dáša DOLEŽALOVÁ[4], Petr DVOŘÁK[1, 4, 5], Aleš HAMPL[1, 4, 5]

[1]Faculty of Medicine, Masaryk University, Brno, Czech Republic
[2]Faculty of Informatics, Masaryk University, Brno, Czech Republic
[3]Faculty of Medicine, Masaryk University, Brno, Czech Republic
[4]Institute of Experimental Medicine, Academy of Sciences of the Czech Republic, Prague, Czech Republic
[5]Center for Cell Therapy and Tissue Repair, Charles University, Prague, Czech Republic

Due to their unique properties human embryonic stem cells (hESC) represent a promising tool for regenerative medicine. However, when propagated *in vitro* pluripotent hESC tend to accumulate alterations to their karyotype, which may limit their prospective use in patients. Chromosomal instability of aggressive malignancies is considered to be driven, at least in part, by centrosomal overamplification with extra MTOCs perturbing balanced chromosome segregation. Here we report, for the first time, very high frequency of cells with supernumerary centrosomes in cultures of hESC. This inadequate situation features undifferentiated state of hESC and becomes progressively suppressed upon their prolonged propagation in culture. Furthermore, we demonstate that improved attachement as well as inhibition of key molecular regulators of centrosomal metabolism, CDK2 and Aurora A, diminish frequency of multicentrosomal mitoses. In other words, attenuated cell attachement and deregulation of machinery controlling centrosome numbers both contribute to centrosomal overamplification in hESC. Still, linking the number of centrosomes to the ploidy indicates that not only overduplication within a single cell cycle but also mitotic failure is involved in generation of numerical centrosomal abnormalities in hESC. Our data point to the supernumerary centrosomes as to be another significant risk factor for cultured hESC in terms of genomic integrity maintenance.

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HEAT SHOCK AT 42ºC RESULTS IN ACCUMULATION OF DCP2 AND NGR1 PROTEINS INDEPENDENTLY OF P-BODY SCAFFOLDS

Tomas GROUŠL[1], Pavel IVANOV[2], Ivana FRÝDLOVÁ[1], Ivana MALCOVÁ[1], Lenka NOVÁKOVÁ[1], Jiří HAŠEK[1]

[1]*Institute of Microbiology of AS CR, v.v.i., Prague, Czech Republic*
[2] *A.N. Belozersky Institute of Physico-Chemical Biology MSU, Moscow, Russia*

A passage through all steps of the eukaryotic gene expression is dominantly mediated by proteins, which interact with ribonucleic acids to form into ribonucleoproteins (RNPs). Composition of these RNPs depens on various factors, including the response to heat stress, when the cells reprogram their expression machinery and form specific RNPs. We have recently referred that robust heat shock at 46ºC induces formation of stress granules (SGs) in yeast *Saccharomyces cerevisiae*. These stress granules have specific composition, which reflects SGs structure of higher eukaryotes. However, some typical P-body proteins, such as Dcp2p or Dhh1p, were colocalized with heat shock-induced SGs. The relationship between SGs and P-bodies in heat-stressed yeast *S. cerevisiae* is poorly understood. Here we show that a milder heat shock at 42ºC results in alteration of a translational profile and leads to accumulation of Dcp2 and Ngr1 proteins even in the edc3lsm4C deletion mutant, which is unable to form P-bodies. However, upon heat shock at 37°C, in contrast to the wild-type strain, the edc3lsm4C deletion mutant does not form any Dcp2p accumulations. This indicates that accumulations of Dcp2p at 37°C and 42°C utilize alternative routes for their assembly. We suggest that Dcp2p accumulation at 37°C represent P-bodies because of the cells only reprogram their expresion machinery in contrast to Dcp2p accumulations at 42°C, which may have more stabilized structure, e.g. by accumulated Ngr1p.

This work was supported by Research Centre LC545, grant CSF 204/09/1924, and the Institutional Research Concept AV0Z50200510.

CYTOSKELETON AND HEAT-INDUCED STRESS GRANULES IN S. CEREVISIAE

Jiří HAŠEK[1], Tomáš GROUŠL[1], Pavel IVANOV[2], Eva TARANTOVÁ[1], Ivana FRÝDLOVÁ[1], Ivana MALCOVÁ[1]

[1] *Institute of Microbiology AS CR, v.v.i., Prague, Czech Republic*
[2] *A.N. Belozersky Institute of Physico-Chemical Biology MSU, Moscow, Russia*

Cytoskeletal elements have been shown to interact with component of the protein synthetic machinery. However, the physiological significance of these interactions is poorly understood. Recent data show, that disruption of actin filaments, but not microtubules, leads to a major reduction in protein synthesis. When translation initiation is impaired, cytoplasmic ribonucleoproteins are observed to accumulate in stress granules (SGs).

We present here that in *Saccharomyces cerevisiae* cells heat-shocked at 46°C the assembly of SGs is independent on integrity of F-actin cytoskeleton or microtubules. We also observed a partial colocalization of SGs with alpha-tubulin but not with collapsed bundles of F-actin. Nevertheless, SGs were obviously associated with mitochondria. Interestingly, upon a mild heat shock at 42°C F-actin cables were also collapsed. Some elongation factors were also in granules but eIF3a was not accumulated. We suggest that aggregation of F-actin might be a prerequisite of SGs assembly.

This work was supported by Research Centre LC545, grant CSF 204/09/1924, and the Institutional Research Concept AV0Z50200510.

COMPUTATIONAL MODELLING OF MECHANICAL TESTS OF CELLS

Petr SŮKAL, Jiří BURŠA

Institute of Solid Mechanics, Mechatronics and Biomechanics, Faculty of Mechanical Engineering, Brno University of Technology, Brno, Czech Republic

There are many evidences that some physiological and pathological changes in the arterial wall (atherosclerosis, tissue remodelation, etc.) are initiated on the cell level and are influenced by the response of cells to their mechanical loading. This transformation is called mechanotransduction. It depends on the mechanical properties of individual cells, specifically their components (cytoskeleton, nucleus, etc.). If we want at least try to understand these processes, it is necessary to approach the problem of inter-disciplinary and to collaborate with experts from various branches of science.
This work deals with the computational modeling of mechanical behavior of isolated smooth muscle cells of artery walls. The presentation brings an overview of computational models solved in the Institute of Solid Mechanics, Mechatronics and Biomechanics. We started with the simplest model, in which the cell is modeled as a homogeneous isotropic continuum only, and continued with more complex models consisting of several parts to the most complex structural model, which includes cytoplasm, membrane, nucleus and cytoskeleton consisting of nuclear elements, membrane elements and connecting elements.

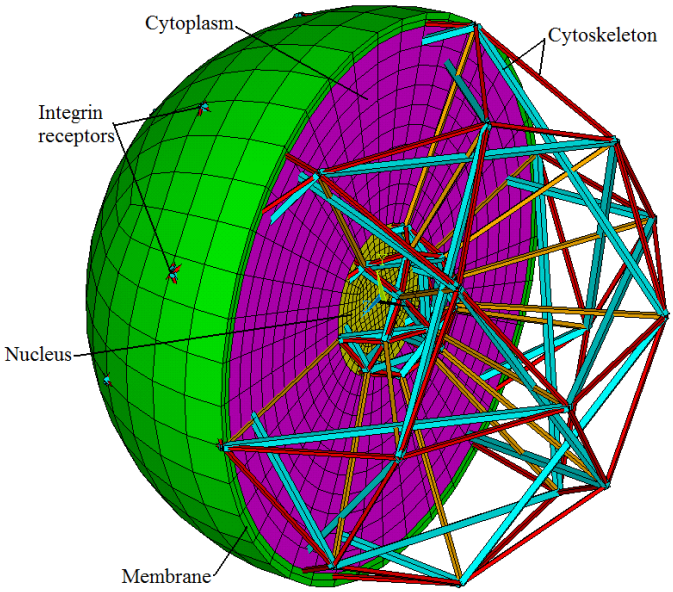

Fig. 1 Finite element mesh of the structural model of the cell

Computational modeling can help us to understand better the transmission of mechanical load from the cell surrounding into the nucleus. Understanding this is one of the prerequisites for investigating the biochemical response to specific mechanical stimuli.
The tensile test simulation was chosen from the amount of mechanical tests performed with the individual cells on the basis of available experimental results presented in literature.

LIST OF PARTICIPANTS

Beránek Karel	Ústav molekulární genetiky AVČR Vídeňská 1083 142 20 Praha 4	karel.beranek@img.cas.cz
Binarová Pavla	Mikrobiologický ústav AVČR Vídeňská 1083 142 20 Praha 4	binarova@biomed.cas.cz
Bišová Kateřina	Mikrobiologický ústav AVČR Novohradská 237 379 81 Třeboň	bisova@alga.cz
Böhmová Tereza	Ústav živočišné fyziologie a genetiky AVČR Rumburská 89 277 21 Liběchov	bohmova@iapg.cas.cz
Bokvaj Pavel	Katedra experimentální biologie rostlin PřF UK Viničná 5 128 44 Praha	pavel@bokvaj.cz
Cenklová Věra	Ústav experimentální botaniky AVČR Sokolovská 6 772 00 Olomouc	cenklova@ueb.cas.cz
Cupák Ladislav	Ústav molekulární genetiky AVČR Vídeňská 1083 142 20 Praha 4	ladislav.cupak@img.cas.cz
Černohorská Markéta	Ústav molekulární genetiky AVČR Vídeňská 1083 142 20 Praha 4	marketa.cernohorska@img.cas.cz
Čížková Mária	Mikrobiologický ústav AVČR Novohradská 237 37 981 Třeboň	majka@alga.cz
Doskočilová Anna	Mikrobiologický ústav AVČR Vídeňská 1083 142 20 Praha 4	Doskocilovaa@seznam.cz
Dráber Pavel	Ústav molekulární genetiky AVČR Vídeňská 1083 142 20 Praha 4	pavel.draber@img.cas.cz
Dráber Petr	Ústav molekulární genetiky AVČR Vídeňská 1083 142 20 Praha 4	draberpe@img.cas.cz
Dráberová Eduarda	Ústav molekulární genetiky AVČR Vídeňská 1083 142 20 Praha 4	eduarda.draberova@img.cas.cz
Dráberová Lubica	Ústav molekulární genetiky AVČR Vídeňská 1083 142 20 Praha 4	draberlu@img.cas.cz

LIST OF PARTICIPANTS

Fendrych Matyáš	Ústav experimentální botaniky AVČR Rozvojová 263 165 02 Praha 6 – Lysolaje	fendryc1@natur.cuni.cz
Frýdlová Ivana	Mikrobiologický ústav AVČR Vídeňská 1083 142 20 Praha 4	frydlova@biomed.cas.cz
Groušl Tomáš	Mikrobiologický ústav AVČR Vídeňská 1083 142 20 Praha 4	grousl@biomed.cas.cz
Hašek Jiří	Mikrobiologický ústav AVČR Vídeňská 1083 142 20 Praha 4	hasek@biomed.cas.cz
Hlavová Monika	Mikrobiologický ústav AVČR Novohradská 237 379 81 Třeboň	monika.hlavova@gmail.com
Holubcová Zuzana	Biologický ústav LFMÚ Kamenice 5 625 00 Brno	zholub@med.muni.cz
Janisch Roman	Biologický ústav LFMU Kamenice 5, A7 625 00 Brno	rjanisch@med.muni.cz
Kobrlová Jana	Katedra experimentální biologie rostlin PřF UK Viničná 5 128 44 Praha	akcni.krtecek@post.cz
Kočárová Gabriela	Mikrobiologický ústav AVČR Vídeňská 1083 142 20 Praha 4	Kocarova.Gabriela@seznam.cz
Kohoutová Lucie	Mikrobiologický ústav AVČR Vídeňská 1083 142 20 Praha 4	lucie.kohoutova@volny.cz
Kourová Hana	Mikrobiologický ústav AVČR Vídeňská 1083 142 20 Praha 4	kourova@post.cz
Kukla Jakub	Katedra experimentální biologie rostlin PřF UK Viničná 5 128 44 Praha	kukla.jakub@seznam.cz
Malcová Ivana	Mikrobiologický ústav AVČR Vídeňská 1083 142 20 Praha 4	janatova@biomed.cas.cz
Matoušková Jindřiška	Vysoká Škola Chemicko-Technologická Technická 5 166 28 Praha 6	jinmat@centrum.cz

LIST OF PARTICIPANTS

Mayer Alexandra	Ústav živočišné fyziologie a genetiky AVČR Rumburská 89 277 21 Liběchov	mayer@iagp.cas.cz
Pačes Jan	Ústav molekulární genetiky AVČR Vídeňská 1083 142 20 Praha 4	jan.paces-jevansky@img.cas.cz
Pleskot Roman	Ústav experimentální botaniky AVČR Rozvojová 263 165 02 Praha 6 – Lysolaje	pleskot@ueb.cas.cz
Richterová Věra	Ústav molekulární genetiky AVČR Vídeňská 1083 142 20 Praha 4	vera.richterova@img.cas.cz
Sůkal Petr	Fakulta strojního inženýrství VUT Technická 2896/2 616 69 Brno	petr.sukal@gmail.com
Sulimenko Vadym	Ústav molekulární genetiky AVČR Vídeňská 1083 142 20 Praha 4	vadym.sulimenko@img.cas.cz
Šolc Petr	Ústav živočišné fyziologie a genetiky AVČR Rumburská 89 277 21 Liběchov	solc@iapg.cas.cz
Tomaštíková Eva	Ústav experimentální botaniky AVČR Sokolovská 6 772 00 Olomouc	eva.tomastikova@seznam.cz
Urban Edit	Institute of Molecular Biotechnology Austrian Academy of Sciences Dr. Bohrgasse 3. A -1030 Vienna	edit.urban@imba.oeaw.ac.at
Varga Vladimír	Sir William Dunn School of Pathology University of Oxford South Parks Road OX1 3RE Oxford	vladimir.varga@path.ox.ac.uk
Vinopal Stanislav	Ústav molekulární genetiky AVČR Vídeňská 1083 142 20 Praha 4	stanislav.vinopal@img.cas.cz
Vítová Milada	Mikrobiologický ústav AVČR Novohradská 237 37 981 Třeboň	vitova@alga.cz
Volc Jindřich	Mikrobiologický ústav AVČR Vídeňská 1083 142 20 Praha 4	volc@biomed.cas.cz

LIST OF PARTICIPANTS

Vosolsobě Stanislav	Katedra experimentální biologie rostlin PřF UK Viničná 5 128 44 Praha	vosolsob@natur.cuni.cz
Widlund O. Per	Max Planck Institute of Molecular Cell Biology and Genetics Pfotenhauerstrasse 108 01307 Dresden	widlund@mpi-cbg.de
Zachleder Vilém	Mikrobiologický ústav AVČR Novohradská 237 37 981 Třeboň	zachleder@gmail.com

FIRM REPRESENTATIVES

Basíková Hana	Eppendorf Czech & Slovakia s.r.o. Kolovratská 1476 251 01 Řičany u Prahy	hana.basikova@eppendorf.cz
Horváth Viktor	ROCHE s.r.o. Dukelských hrdinů 52 170 00 Praha 7	viktor.horvath@roche.com
Nováková Lucie	M.G.P. spol. s.r.o Kvítková 1575 760 01 Zlín	novakova@mgp.cz
Pavlíček Jiří	KRD s.r.o. Pekařská 12 155 00 Praha 5	jiri.pavlicek@krd.cz
Pop Tomáš	OLYMPUS CZECH GROUP, s.r.o. Evropská 176/16 160 41 Praha 6	pop@olympus.cz
Steinbauerová Anna	EXBIO Praha, a.s. Nad Safinou II 366 252 42 Vestec	steinbauerova@exbio.cz
Škrdla Pavel	Scintila, s.r.o. Mahlerova 25 586 01 Jihlava	skrdla@scintila.cz
Šušla Martin	Sigma-Aldrich spol. s r.o. Sokolovská 100/94 186 00 Praha 8	martin.susla@sial.com
Tichá Olga	SCHOELLER PHARMA, s.r.o Vídeňská 1398/124 148 00 Praha 4	Olga.Ticha@schoeller.cz

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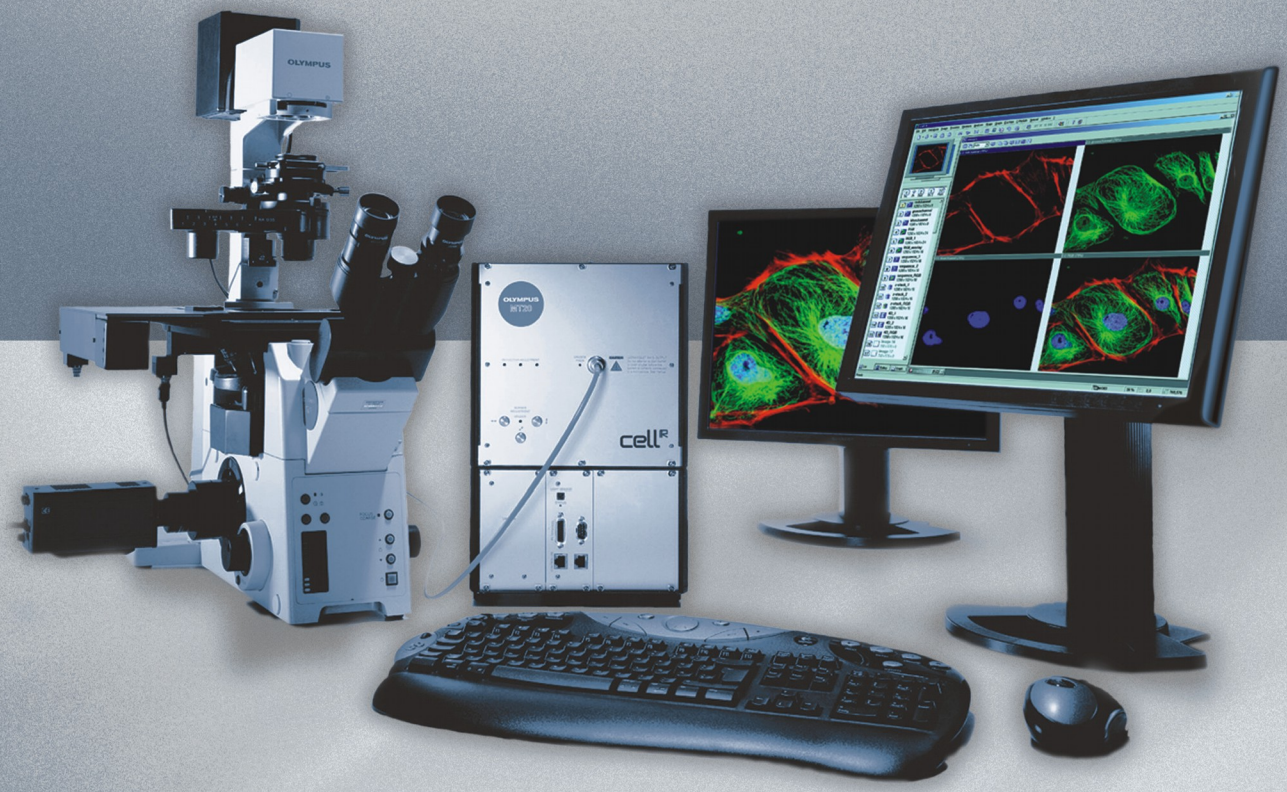

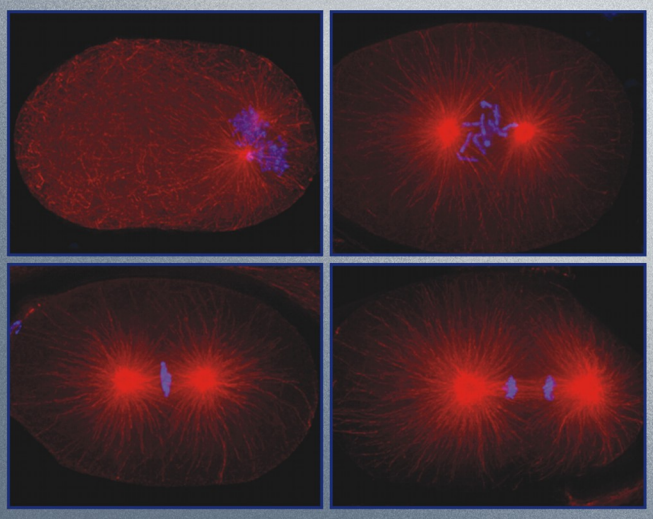

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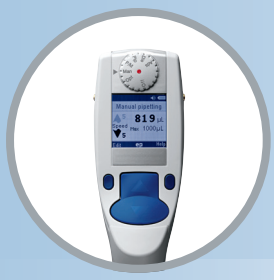

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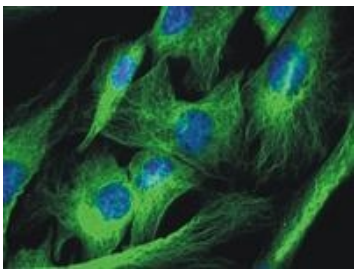

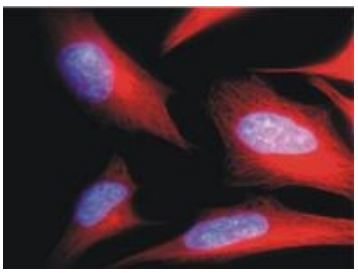

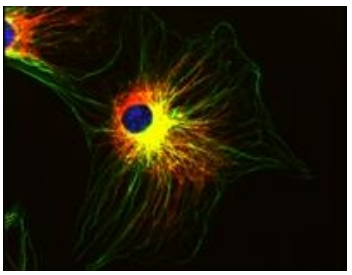

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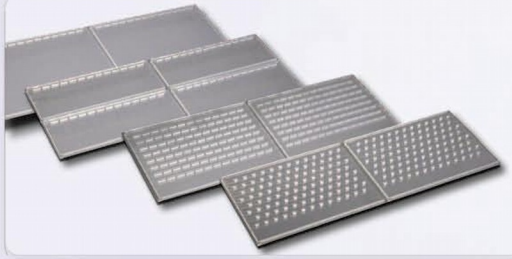

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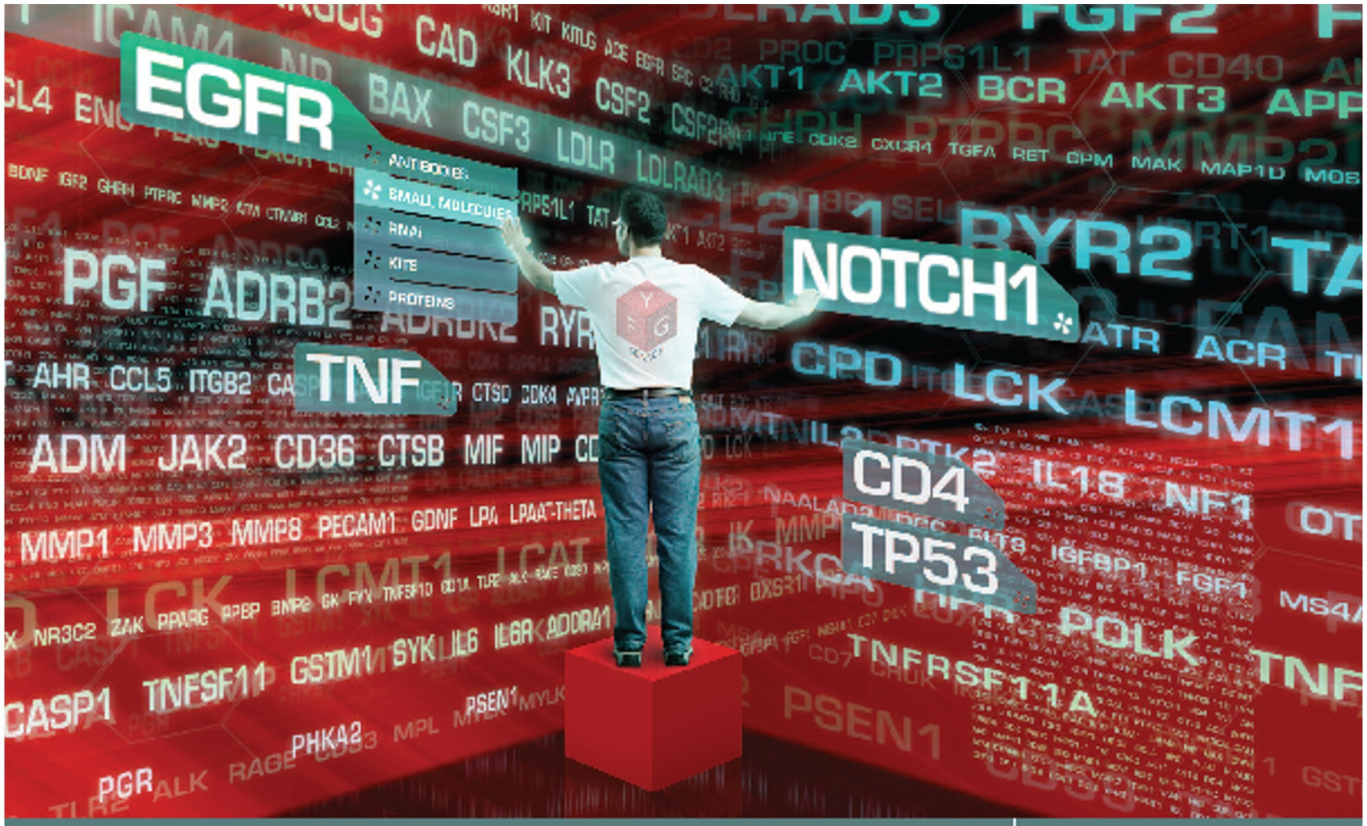

INNOVATION @ WORK

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