

experiments macrophages showed slightly higher preference for engulfing apoptotic cells and apoptotic cells were similarly effective competitors as necrotic cells. We found that prefeeding of macrophages with either apoptotic or necrotic cells does not influence subsequent phagocytic preference. Our result might provide insights into the so far not studied aspects of necrotic cell uptake. Funding: OTKA K 104228 to Zsuzsa Szondy and Internal research grant of University of Debrecen to Zsolt Sarang.

P9

Biochemical activities of the Wiskott-Aldrich syndrome homology region 2 domains of Sarcomere Length Short protein

Mónika Ágnes Tóth¹, Péter Gaszler¹, Judit Viktória Fórizs¹, Andrea Teréz Vig¹, Ede Migh², József Mihály², Beáta Bugyi¹

¹Medical School, Department of Biophysics, Pécs; ²Institute of Genetics, Hungarian Academy of Sciences, Biological Research Centre, Szeged

Drosophila melanogaster Sarcomere Length Short (SALS) is a recently identified Wiskott - Aldrich syndrome protein homology 2 (WH2) domain protein involved in skeletal muscle thin filament regulation. SALS was shown to be important for the establishment of the proper length and organization of sarcomeric actin filaments. Here we present the detailed characterization of the biochemical activities of the tandem WH2 domains of SALS (SALS-WH2). Our results revealed that SALS-WH2 binds both monomeric and filamentous actin and shifts the monomer : filament equilibrium towards monomeric actin. In addition, SALS-WH2 can bind to but fails to depolymerize phalloidin-, or jasplakinolide-bound actin filaments. These interactions endow SALS-WH2 with two major activities in the regulation of actin dynamics: SALS-WH2 sequesters actin monomers into non-polymerizable complexes and enhances actin filament disassembly by severing, which is modulated by tropomyosin. We also show that profilin does not influence the activities of the WH2 domains of SALS in actin dynamics. In conclusion, the tandem WH2 domains of SALS are multifunctional regulators of actin dynamics. Our findings suggest that the activities of the WH2 domains do not reconstitute the presumed biological function of the full-length protein. Consequently, the interactions of the WH2 domains of SALS with actin must be tuned in the cellular context by other modules of the protein and/or sarcomeric components for its proper functioning. This research was supported by grants from the Hungarian Science Foundation OTKA K109689 (to BB) and from the National Innovation Office „Baross Gábor” Program (REG-DD-09-1-2009-0009 Tírfm 09 (to BB)). This research was supported by the European Union and

the State of Hungary, co-financed by the National Social Fund in the framework of TAMOP-4.2.1-11-1-2012-0001 National Excellence Program.

P10

Intramolecular interactions regulate the binding of the scaffold protein Tks4

Anna Cserkaszkzy¹, László Koprivanacz¹, Balázs Merő¹, Bálint Kudlik¹, Gergő Gógl², László Nyitrai¹

¹Institute of Enzymology, Research Group of Molecular Sciences, Hungarian Academy of Sciences, Budapest; ²Department of Biochemistry, Loránd University, Budapest

The scaffold protein Tks4 is a member of the p47phox-related organizer superfamily and plays a role in cell motility by being essential for the formation of actin-rich membrane protrusions, lamellipodia and invadopodia of normal cells. Tks4 is regulated by the growth factor (EGF) signaling pathway and the translocation of Tks4 from the cytoplasm to the plasma membrane. Tks4 and the related protein p47phox share many features in their N-terminal region: a phosphotyrosine (PX) domain is followed by two SH3 domains (so called „tandem SH3”) and a proline-rich region (PRR). Tks4 contains several other domains and several other PRRs. Intramolecular interactions are essential for the autoinhibited state of p47phox. The PX domain binds the PRR while the SH3 domains interact with the PX domain preventing membrane binding. Based on the conserved features of p47phox and Tks4 and the intramolecular interaction between the PX and the PX domains of Tks4, we hypothesized that Tks4 is also autoinhibited. In this study we aimed to study intramolecular interactions within the PX part of the Tks4 protein that are essential for its presumable autoinhibited conformation. By fluorescence-based titration and X-ray Scattering that the tandem SH3 domains of Tks4 binds the PRR and that the PX domain interacts with the third SH3 domain. Phosphatidylinositol (3)-phosphate (PI3P) and phosphatidylserine (PS) were identified as the main binding partners of the tandem SH3 together with the PRR. PI3P and PS promoted PA binding while PI3P inhibited PS binding. These findings further support the role of Tks4 in cell motility and were supported by OTKA and MedInProt grants.

P20**Dynamic protein structural ensembles based on NMR data: methodology & applications**

Zoltán Gáspári, Bertalan Kovács, Zita Harmat
Pázmány Péter Catholic University, Faculty of Information Technology and Bionics, Budapest

Protein NMR is the most powerful technique to study protein internal dynamics at the atomic level at multiple time scales. Combination of NMR-derived parameters with molecular dynamics calculations can lead to atomic-level ensemble-based structural models that are consistent with the experimentally determined mobility. We have implemented different restraining schemes in GROMACS [1, 2] and performed calculations on different proteins for which experimental data are available [3, 4]. The obtained ensembles can be used to understand ligand binding and can also yield clues of selected catalytic mechanisms.

[1] Hess B, Kutzner C, van der Spoel D, Lindahl E. (2008) GROMACS4: algorithms for highly efficient, load-balanced, and scalable molecular simulation. *J Chem Theor Comp* 4: 435-47.

[2] Fizil Á, Gáspári Z, Barna T, Marx F, Batta G. (2015) „Invisible” conformers of an antifungal disulfide protein revealed by constrained cold & heat unfolding, CEST-NMR experiments and molecular dynamics calculations *Chem Eur J* 21: 5136-44.

[3] Heikkinen O. et al. (2009) Solution structure of the parvulin-type PPIase domain of *Staphylococcus aureus* PrsA – Implications for the catalytic mechanism of parvulin. *BMC Struct Biol* 9: 17.

[4] Horváth G. et al. (2016) Structural determinants of ligand binding in the ternary complex of human ileal bile acid binding protein with glycocholate and glycochenodeoxycholate obtained from solution NMR. *FEBS J* 283: 541-55.

P21**Coordination of actin-microtubule dynamics by DAAM formin**

Péter Gaszler, Judit Viktória Fórizs, Andrea Teréz Vig, Beáta Bugyi

¹ Department of Biophysics, Medical School, University of Pécs, Pécs

The proper functioning of cells relies on the dynamic remodeling of their actin and microtubule cytoskeleton. Recent studies indicate that these cytoskeletal elements do not function individually, but their structure and dynamics are co-regulated by associated proteins. DAAM, the member of the formin family protein has a well-established role in actin dynamics regulation. It possesses two conserved formin homology domains (FH1-FH2), which catalyze actin nucleation and processively mediate filament elongation. In this work, we analyzed the possible role of DAAM in the coordination of actin-

microtubule cytoskeleton by fluorescence spectroscopy, TIRF microscopy and protein biochemical approaches. We found in centrifugation-based sedimentation experiments, that DAAM can organize individual actin filaments, as well as microtubules into higher-order bundled structures. Interestingly, we found that DAAM can physically link together the actin and microtubule networks by visualizing single polymers in TIRF microscopy. We also investigated the importance of the different regions of DAAM in these activities. We showed that the conserved FH2 domain is sufficient and necessary for actin bundling, but it fails to bundle microtubules. The C-terminal elements, downstream to the FH domains are essential for microtubule bundling and the co-regulation of the two polymer networks. In conclusion, we identified a novel role of DAAM as a possible regulator of the functional coordination between the actin and microtubule cytoskeleton.

P22**Size-Dependent Impact of Silver Nanoparticles on Multidrug Resistant Breast Cancer Cells**

Mohana Krishna Gopisetty¹, Dávid Kovács¹, Nóra Igaz¹, Zoltán Kónya^{2,3}, Imre Miklós Boros^{1,4}, Mónika Kiricsi¹

¹Department of Biochemistry and Molecular Biology, University of Szeged, Szeged; ²Department of Applied and Environmental Chemistry, University of Szeged, Szeged; ³MTA-SZTE Reaction Kinetics and Surface Chemistry Research Group, Szeged; ⁴Institute of Biochemistry, Biological Research Centre, Hungarian Academy of Sciences, Szeged

Multidrug resistance (MDR) is defined as the ability of cancerous cells to evade structurally and functionally unrelated chemotherapeutic drugs, which phenomenon renders the general tumor therapy regimens ineffective. MDR is largely attributed to the overexpression of P-glycoprotein (Pgp) [1], a membrane localized ATP-driven efflux protein, member of the ATP-binding cassette (ABC) transporter family. Previous findings of our research group suggested the possible role of approximately 30 nm sized silver nanoparticles (AgNPs) in inhibiting ABC transporters in multidrug resistant Colo 320 colon cancer cells [2]. Therefore, the present work was designed to elucidate the size-dependent impact of AgNPs on Pgp overexpressing drug resistant MCF7-KCR breast cancer cells. Citrate coated quasi-spherical AgNPs of three different sizes (approx. 4, 17 and 72 nm) have been synthesized and characterized by electron microscopy, dynamic light scattering and UV/VIS absorption. Their toxicity, Pgp inhibiting activity and the underlying molecular mechanisms have been examined in MCF7-KCR cells by viability assays, Western blotting and by

P69

The CT region of DAAM has a supporting role in FH2-mediated actin dynamics regulation

Péter Gaszler¹, Andrea Vig¹, Judit Viktória Főrizs¹, Mónika Ágnes Tóth¹, István Földi², József Mihály², Beáta Bugyi¹

¹University of Pécs, Medical School, Department of Biophysics, Pécs; ²Institute of Genetics, Biological Research Centre, Hungarian Academy of Sciences, Szeged

DAAM is a DRF formin protein essential for the regulation of actin cytoskeleton dynamics in diverse biological processes. The conserved FH1-FH2 domains of DAAM catalyze actin nucleation and processively mediate filament elongation. These activities of FH2 are autoregulated by the N- and C-terminal regions. The C-terminal DAD-CT of formins was suggested recently to directly regulate actin dynamics, as well. We analyzed the role of DAD-CT region of *Drosophila* DAAM in its interaction with actin by in vitro biochemical and in vivo genetic approaches. We found that DAD-CT can bind actin and enhance the nucleating activity of FH2, through the CT region. Despite of being able to bind actin, DAD-CT does not significantly influence actin assembly in the absence of the FH2 domain. Analysis of the I732A loss-of-function mutation in FH2 and FH2-DAD-CT revealed that DAD-CT can compensate for the compromised actin-binding of the FH2 domain. However, the actin nucleation, elongation and barbed end capping activity of the FH2 domain can not be restored by the presence of DAD-CT. Consistently, in vivo data show that the CT region is not essential, yet contributes to DAAM-mediated filopodia formation in primary neurons. In conclusion, DAAM DAD-CT possesses an FH2-dependent function in actin dynamics. Our data suggest that the FH2 region is the core actin-interacting element of DAAM and DAD-CT has only a supporting role in actin nucleation, presumably through stabilization of nucleation intermediates. Supported by grants from the Hungarian Science Foundation (OTKA) Grants K109689 (to BB), K109330 (to JM) and by the National Innovation Office „Baross Gábor” Program (REG-DD-09-1-2009-0009 Tírfm 09 (to BB)). This research was supported by the European Union and the State of Hungary, co-financed by the European Social Fund in the framework of TÁMOP 4.2.4.A/2-11-1-2012-0001 „National Excellence Program”.

P70

Decreased PMCA4b levels in the erythrocyte membrane of healthy individuals with a specific PMCA4b haplotype in the ATP2B4 gene

Boglárka Zámboi¹, Edit Szabó², Adrienn Nemeth¹, László Sarkadi^{1,2}

¹Institute of Enzymology, Hungarian Academy of Sciences, Budapest; ²Department of Medical and Health Sciences, Semmelweis University, Budapest; ³SE Molecular Biophysics Research Group, Department of Biophysics and Biotechnology, Semmelweis University, Budapest

Aims: We have developed a novel method to measure quantitatively the levels of membrane proteins in red blood cells (RBCs). We have screened the PMCA4b levels in a large number of healthy individuals and found a significant heterogeneity in the levels. We wished to compare the functional activity and find the potential genetic variants that lead to reduced PMCA4b levels. In subjects with low PMCA4b levels, we performed a blot analysis of the RBC membrane. It revealed that the molecular mass of the protein was unchanged and no compensation by other PMCA4b variants. To explore the reduced PMCA4b levels, we performed a calcium pump function in RBCs. In order to explore the genetic variants, we performed a reduced expression, we performed a gene in individuals with low PMCA4b levels. Interestingly, we found no alteration in the acid changes, while we found a variant in part of the gene, consisting of 20 bp. TaqMan based genotyping, we performed a screen a larger cohort, and found a variant in PMCA4b protein level in RBCs. The minor variant of this haplotype is associated with a reduced PMCA4b level (p<0.001; MAF=0.1; CT 28%).

Conclusions: We found a haplotype in the ATP2B4 gene which causes significantly reduced levels in the RBC membrane. This haplotype is overlapping the recently described variant of the ATP2B4 gene, and decreased expression is probably related to alteration of transcription factors in this region. While we found these alterations in healthy adults, recent GWA studies indicated that this haplotype result in a decreased haemoglobin concentration in RBCs, and may be protective against anemia [3]. **Acknowledgment:** This work was supported by OTKA K115375.

[1] Sarkadi B, et al. (2013) Quantitative analysis of the erythrocyte membrane. *Cell* 154: 1023-1032. EP2839293 A2.