



the rate of the formation of the vanadate trapped post-hydrolytic complex in accordance with the substrate stimulation of the ATPase activity of the protein. We also found that the binding of 5D3 antibody to ABCG2 is reversible; the dissociation rate of 5D3 is enhanced by ATP/Mg²⁺ and substrates due to the function dependent conformational changes of the transporter.

Based on the above data our novel 5D3 antibody based assays are applicable for studying the details of the catalytic cycle of ABCG2.

Supported by: TÁMOP 4.2.4.A/2-11-1-2012-0001; OTKA grants PD75994 and K72762, Szodoray fellowship (Katalin Goda) and TÁMOP 4.2.2.A-11/1/KONV-2012-0023 "VÉD-ELEM" project.

Keywords: ABCG2, 5D3 monoclonal antibody, conformation changes

P-45

THE ACTIVITIES OF THE DISHEVELED-ASSOCIATED ACTIVATOR OF MORPHOGENESIS (DAAM) FORMIN IN ACTIN DYNAMICS

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

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

Disheveled-associated activator of morphogenesis (DAAM) is a Diaphanous-related formin protein essential for the regulation of actin cytoskeleton dynamics in diverse biological processes. DAAM plays a role in organizing apical actin cables that define the tracheal cuticle pattern, it is required for axonal growth and guidance by promoting filopodia formation in the growth cone, as well as for sarcomerogenesis. DAAM consists of conserved domains including the canonical Formin Homology (FH) FH1 and FH2 domains, which are flanked by N-, and C-terminal elements.

To better understand the molecular mechanisms supporting the biological functions of DAAM, our aim was to dissect the *in vitro* physico-chemical activities of each of its domains.

We found that the FH2 domain interacts with actin and enhances actin assembly by catalyzing nucleation. The FH1 domain interacts with profilin. By processively associating to filament barbed ends the FH1-FH2 influences filament elongation in a profilin-gated manner. These activities of the FH1-FH2 are autoregulated by the intramolecular interactions between the N-terminal Diaphanous Inhibitory Domain (DID) and the C-terminal Diaphanous Autoregulatory Domain (DAD). We showed that DAAM and Capping Protein (CP) can tune filament growth in an antagonistic fashion. We also identified the very C-terminal CT region of DAAM as a novel actin binding site. Despite of being able to bind actin isolated CT does not influence actin dynamics on its own. However, it can tune the nucleating activity and the filament end interaction properties of DAAM in an FH2 domain dependent manner.



In conclusion, the core actin interacting element of DAAM is the FH2 domain, but its activities are regulated in the biological context by both internal (DID, DAD, CT regions) and external factors (profilin, CP).

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'. '„Supported BY the ÚNKP-16-4-III New National Excellence Program of the Ministry of Human Capacities"

Keywords: actin, formin, DAAM, *Drosophila*



P-46

COORDINATION OF ACTIN-MICROTUBULE DYNAMICS BY DISHEVELED-ASSOCIATED ACTIVATOR OF MORPHOGENESIS (DAAM) FORMIN

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

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

The crosstalk between the actin and microtubule cytoskeleton is essential for diverse cellular functions, including cell motility, division, adhesion and intracellular trafficking. The dynamic and structural features of these cytoskeletal elements are tightly co-regulated by associated proteins, however, the underlying mechanisms are still elusive.

Disheveled-Associated Activator of Morphogenesis (DAAM), the member of the formin family protein has an established role in actin dynamics regulation. DAAM consists of conserved domains including the canonical Formin Homology (FH) FH1 and FH2 domains, which catalyze actin nucleation and processively mediate filament elongation. The activities of the FH domains in actin assembly are regulated by N-, and C-terminal elements.

In this work, we analyzed the possible role of DAAM in the co-regulation of actin-microtubule dynamics by *in vitro* protein biochemical and biophysical approaches.

We found that DAAM can organize individual actin filaments, as well as microtubules into higher-order bundled structures. Moreover, we found that DAAM can co-align the two filament systems. We also investigated the importance of the different regions of DAAM in these activities. We showed that the 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-alignment of the two polymer networks.



In conclusion, our results reveal a novel role of DAAM as a possible regulator of the functional coordination between the actin and microtubule cytoskeleton.

This research was supported by grants from the Hungarian Science Foundation (OTKA) Grants K109689, "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", "Supported by the ÚNKP-16-4 New National Excellence Program of the Ministry of Human Capacities", "Supported by the ÚNKP-16-2 New National Excellence Program of the Ministry of Human Capacities"

Keywords: Actin, microtubule, formin

P-47

BIOCHEMICAL CHARACTERIZATION OF MYOSIN 16 DOMAINS

András Kengyel^{1,2}, Elek Telek¹, Zoltán Kónya⁴, Bálint Bécsi⁴, Ferenc Erdődi⁴, Miklós Nyitrai^{1,2,3}

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

² János Szentágothai Research Center, Pécs, Hungary

³ MTA-PTE Nuclear-Mitochondrial Interactions Research Group, Pécs, Hungary

⁴ Department of Medical Chemistry, University of Debrecen, Debrecen, Hungary

The unconventional myosin 16b (Myo16b), which may have a role in the neuronal development and the regulation of cell cycle, can be found both in the nucleus and in the cytoplasm. Myo16b contains a motor domain, a short neck region, an intrinsically disordered tail (My16Tail) and an ankyrin-repeat containing N-terminal domain, called the ankyrin domain (My16Ank). Ankyrin repeats are present in several other proteins, e.g. in the regulatory subunit (MYPT1) of the myosin phosphatase holoenzyme, which binds to the protein phosphatase-1 catalytic subunit (PP1c) and My16Ank shows a sequence similarity to MYPT1.

In our present work we are attempting to characterize the Myo16b domains: the My16Ank, the motor domain, and the My16Tail *in vitro* using recombinant protein fragments.

We tested the effects of My16Ank on the myosin motor function using skeletal muscle myosin as a model system. The results showed that My16Ank bound to skeletal muscle myosin and the actin-activated ATPase activity of the skeletal myosin was increased in the presence of My16Ank. Besides, we showed, that My16Ank strongly bound to PP1 α and PP1 δ isoforms with submicromolar affinity.

The prolin-rich My16Tail is supposed to bind profilin, but we found no direct interaction between My16Tail and fluorescent profilin-1 using steady-state and time resolved anisotropy measurements. Meanwhile, we also tested the interaction between My16Tail and My16Ank domains, which showed a moderate affinity ($K_D \sim 13 \mu M$).

Our results suggest that one function My16Ank is probably to regulate the motor function of Myo16. It may influence the motor activity and in complex with the PP1c isoforms, it can play