Ph.D. thesis

The activities of the Wiskott-Aldrich syndrome homology region 2 domains of the Sarcomere length short protein in the regulation of actin dynamics

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INTRODUCTION

Model of the assembly of the sarcomeric thin filament array

Sarcomeres are the basic contractile units of striated muscles. The structural features and the functioning of sarcomeres are well resolved, but the molecular mechanism underlying sarcomerogenesis; specifically the assembly, maturation and maintenance of the actin-based thin filament array is one of the major open issues in muscle research. There are several models of sarcomeric thin filament assembly. According to a recent study, a 3-phase model can be supposed based on the sequence of actin incorporation into thin filaments [1]:

Phase 1: In the initial period of sarcomerogenesis an extensive, burst *de novo* actin filament polymerization can be observed that generates a store of homogeneous, almost parallel microfilament array.

Phase 2: The *de novo* polymerization is followed by an interim period of 'patchy' actin incorporation during which individual, uniform sarcomeric units are defined. This mode occurs mainly at the ends of the array; in proximity to the Z-disc, as well as towards the opposite M-line boundary of the nascent array.

Phase 3: The late period is characterized by three distinct modes of actin incorporation: as a consequence of pointed-end elongation the thin filament array continues to extend laterally; radial actin association results in the 'thickening' of the sarcomeres; Z-disc associated actin dynamics contributed to barbed-end turnover.

These modes of the sarcomeric thin filament assembly reflect the mechanisms of spontaneous actin filament organization *in vitro*: *de novo* nucleation and elongation, longitudinal endwise (annealing) and transversal sidewise (bundling/crosslinking) association of existing filaments. Importantly, while actin dynamics in non-muscle cells and in the cell-free environment is dominated by barbed end dynamics, elongation of sarcomeric thin filaments at pointed ends can be observed.

The structural and dynamic features of the phases of thin filament dynamics corresponding to different developmental stages are well distinguished, but the underlying molecular mechanisms are largely unknown.

Actin-associated proteins regulating the formation and dynamics of actin filaments during striated muscle development

The assembly and organization of thin filaments during sarcomerogenesis exhibit a complex spatio-temporal sequence and are manifested through the coordinated interplay of actin-associated proteins [1-3].

The monomer-binding proteins thymosin β4 (Tβ4) and profilin are expressed in the early stages of striated muscle development in vertebrates [4, 5]. These proteins are expected to suppress actin polymerization based on their biochemical activities [6]. They may play a role in the maintenance of the sarcomeric actin monomer pool (sequestration, nucleotide exchange). Several groups of proteins coordinate the dynamics of actin filaments, once they have been formed. The dynamics at actin filament ends is limited by capping proteins [4, 5, 7]. The barbed end is capped by CapZ at the Z-disc, which inhibits elongation, but it also has a role in organizing the filaments in a regular array. The pointed end dynamics is limited by the capping activity of tropomodulin (Tmod). The activity of Tmod is enhanced by its interaction with tropomyosin (Tpm). Different families of proteins were identified which, when purified, catalyze *de novo* filament formation, called assembly promoting factors (APFs). APFs are also expressed in striated muscles [3, 4, 8]. Formins (FHOD, FMNL, Dia, INF, DAAM) and the WH2 domain protein Leiomodin (Lmod) have been identified as members of the APF protein family in muscles. Based on their biochemical activities the attractive hypothesis emerged that formins and Lmods are the *de novo* actin filament nucleators in the sarcomere [8, 9]. However, this function has not been proven *in vivo*. Based on animal model studies formins and Lmods may play a role in the organization of thin filament structures, and not in the initial stage of myofibrillogenesis.

WH2 domain proteins

The WH2 (*Wiskott-Aldrich syndrome homology region 2*) domains are short (20 – 50 aa) actin-binding protein modules [6, 10, 11]. The central element of the domain is an LKKT/V consensus motif. This motif is flanked by an N-terminal amphipathic α-helix followed by an FxxxK linker and a C-terminal region with variable length and composition. When uncomplexed, the isolated WH2 domain is a short, structurally intrinsically disordered region (IDR) in solution, which shows partial folding upon binding to actin [12, 13]. WH2 can occur as a single module or as tandem regions (2 – 4 domains) or in combination with other actin-binding domains [14, 15]. WH2 domain proteins can interact with both actin monomers and filaments. Consequently, they can possess versatile functions in the regulation of actin dynamics depending on the number, the length and the specific sequence of the domains. The WH2 domain protein family emerged as important regulators of muscle actin structures, as well. In skeletal muscles two WH2 domain proteins have been implicated in the regulation of assembly and organization of sarcomeric actin filaments: the vertebrate-specific Leiomodin (Lmod) and the *Drosophila melanogaster* Sarcomere Length Short (SALS) proteins [1, 9, 16, 17]. Although, Lmod and SALS have similar functional properties based on *in vivo* studies, genetic connection between them is unknown [1, 4, 8].

Leiomodin

Leiomodin isoforms (Lmod1 – 3) belong to the tropomodulin protein family. Lmod2 and Lmod3 are present in the heart and skeletal muscles, respectively and play an important role in the organization and length regulation of sarcomeric actin filaments [8]. The dysfunctioning of Lmods is implicated in human diseases (Lmod2: dilated cardiomyopathy [18, 19], Lmod3: nemaline myopathy [16]). Lmod contains two actin (ABS1, ABS2) and one tropomyosin binding sites (TMBS1). A proline-rich region (PRD) and a WH2 domain are found at the C-terminus. Interaction between the PRD and profilin has not been demonstrated. Lmod can catalyze *de novo* actin filament assembly *in vitro* via the synergic effect between the ABS2 and the WH2 domain. The binding of tropomyosin can enhance its nucleation activity. However, the molecular mechanisms underlying the biological function of Lmod are unknown.

Sarcomere length short

The *Sarcomere length short* (*sals*) gene was identified in *Drosophila* primary muscle cells in 2007 [17]. SALS exists in two isoforms; the long (935 aa, *l*SALS) is expressed specifically in muscle, while the short one (533 aa, *s*SALS) can be found in non-muscle tissues. SALS is a *Drosophila* specific protein, its vertebrate orthologue has not been identified yet [4]. *l*SALS possesses two tandem WH2 domains (WH2-1: 399-416 aa, WH2-2: 447-481 aa) and an upstream proline-rich region, while *s*SALS covers the C-terminal half of the long isoform containing only a single WH2 domain and lacking the proline-rich element. Besides these domains, another specific region has not been identified in the protein [17].

SALS exhibits a complex localization pattern during sarcomerogenesis. In *Drosophila* primary muscle cells SALS localizes at the pointed ends of thin filaments while they are elongating in the larval stages. In adults, it redistributes from the pointed ends of full-grown thin filaments to the region flanking the Z line. The mutation of *sals* results in lethality during early embryogenesis, in the larval stages suggesting the essential function of the protein. Loss-of-function studies in *Drosophila* primary muscle cells revealed that *sals* mutant animals have shorter sarcomeres, thinner myofibrils with compromised structural integrity and increased number of sarcomeres as compared to wild-type controls. In contrast, it has no effect on the assembly and organization of myosin II-based thick filaments. Under the electron microscope numerous electron-dense patches were seen, which resembled nemaline bodies characteristic to the severe muscle defect; nemaline myopathy. The gain-of-function analysis of *sals* (*Dmef2-Gal4, UAS-sals*) revealed an aberrant striated pattern and longer sarcomeres. This phenotype was reminiscent to that of observed upon downregulating Tmod. Downregulation of SALS enhanced the effect of Tmod overexpression; this antagonistic effect suggests that SALS and Tmod might genetically interact during thin filament assembly [17]. In a recent study, temporally

controlled silencing of the *sals* gene in young animals (50 h after egg laying) significantly inhibited pointed-end elongation of thin filaments but did not influence the peripheral thickening of sarcomeric thin filament array [1].

Altogether, these results identify SALS as a major actin regulator, specifically mediating pointed-end growth of thin filaments throughout sarcomere maturation. However, the exact interaction between SALS and actin, and its contribution to the biological function of SALS has not been revealed.

AIMS

The structural features and the functioning of sarcomeres are well resolved, but the molecular mechanism underlying sarcomerogenesis; specifically, the assembly, maturation and maintenance of the actin-based thin filament array is one of the major open issue in muscle research. Importantly, while actin dynamics in non-muscle cells and in the cell-free environment is dominated by barbed end dynamics, the elongation of sarcomeric thin filaments seems to be manifested at pointed ends. From this aspect the WH2 domain proteins; the vertebrate Leiomodin (Lmod) and the *Drosophila* specific Sarcomere length short (SALS) can have important roles in the regulation of sarcomeric actin dynamics. Although, genetic relation between Lmod and SALS has not been identified, based on the biological functions their activities may be conserved.

The central interest of my Ph.D. work was the investigation of the molecular mechanisms underlying the biological functioning of SALS. Considering that WH2 domains are known actin-binding motifs, first I sought to describe the biophysical and biochemical aspects of the interactions of the WH2 domains of SALS with actin. Besides the WH2 domains SALS possesses an upstream proline-rich region that can interact with the small actin-binding protein profilin and can affect the SALS-WH2:actin interaction. Therefore, I extended my studies on the role of the proline-rich motif.

The following questions were addressed:

- How does SALS interact with actin?
- How does SALS influence actin dynamics?
- Does SALS have multifunctional features similar to the other WH2 domain-containing proteins?
- Which sarcomeric proteins can influence the effects of SALS on actin dynamics?

For this purpose, I planned to study the interactions of recombinantly produced proteins (SALS-WH2 and SALS-Pro-WH2) with actin monomers and filaments by using *in vitro* protein biochemical and biophysical approaches.

MATERIALS AND METHODS

Protein purification and fluorescence modification

The cDNAs of SALS-WH2 (379 – 531 aa) and SALS-Pro-WH2 (345 – 531 aa) have been cloned into pGEX-2T plasmid and provided by our collaborator (József Mihály, Hungarian Academy of Sciences, Biological Research Centre, Szeged, Hungary). The proteins were purified as Gluthation S-Transferase (GST) fusion proteins from *E. coli* BL21(DE3)pLysS strain (Novagen). Actin and tropomyosin were extracted and purified from acetone-dried rabbit skeletal muscle powder according to standard protocols [20-22]. Human gelsolin (GSN), mouse profilin1 (profilin) and myosin II from rabbit skeletal muscle were purified as described previously [23, 24]. Actin was modified by pyrene (Sigma-Aldrich), IAEDANS (Life technologies), IAF (Sigma-Aldrich), Alexa488®NHS (Invitrogen); tropomyosin and profilin were labeled by Alexa568®C_5 maleimide (Invitrogen) according to standard protocols [20, 25-27].

Fluorescence spectroscopy experiments

The steady-state fluorescence emission and anisotropy of monomeric actin (IAEDANS or Alexa®488NHS labeled G-actin, 1 μM or 20 μM) were measured to study its interaction with the WH2 domains of SALS. The labeled G-actin (1 μM or 20 μM) was incubated with Latrunculin A (4 μM or 20 μM, respectively) to prevent spontaneous polymerization potentially induced by biochemical conditions. Subsequently, SALS constructs were added to the samples. To study the interaction between tropomyosin (A568®C_5-maleimide-Tpm, 1 μM) and SALS anisotropy measurements were performed under different ionic strength conditions (low ionic strength: 10 mM NaCl; medium ionic strength: 50 mM KCl + 10 mM NaCl; high ionic strength: 100 mM KCl + 10 mM NaCl; note that SALS buffer contained 10 mM NaCl). The ionic strength characterizing the strength of the electrostatic interaction between ions was calculated by using the molar concentration and charge of the ions. To derive the dissociation equilibrium constant (K_D) of the SALS:G-actin and SALS:Tpm complexes the SALS (SALS-WH2 or SALS-Pro-WH2) concentration dependence of the fluorescence emission (I), maximum wavelength (λ_{max}) and anisotropy (r) were analyzed. The interaction between SALS and profilin (A568®C_5 maleimide-profilin, 1 μM) was investigated in steady-state anisotropy measurements.

Fluorescence resonance energy transfer (FRET) measurements were performed to determine the stoichiometry of the SALS:G-actin complex. IAEDANS labeled G-actin (1 μM) and IAF labeled G-actin (9 μM) were used as donor (D) as acceptor (A), respectively. The efficiency of the energy transfer between donor and acceptor molecules was derived in G buffer (monomer), F buffer (G buffer + 1 mM $MgCl_2$, 50 mM KCl, filament), in presence of

gelsolin (5 μM, 1 GSN:2 G-actin) or SALS-WH2 (10 μM). The fluorescence emission was corrected for the inner filter effect caused by the self-absorption of the samples.

Different experiments were performed using pyrene labeled actin to study the effects of SALS on the actin monomer:filament equilibrium:

Bulk polymerization kinetics experiments: Polymerization of G-actin (2.5 μM, containing 5 % pyrenyl-actin) was initiated by the addition of 1 mM $MgCl_2$ and 50 mM KCl in the absence and presence of different concentrations of SALS constructs. Actin assembly was measured by monitoring the change in pyrenyl fluorescence emission. The initial rate of actin assembly was derived from the initial slope (0 – 500 s) of the pyrenyl traces and plotted as the function of [SALS]. The half-inhibition concentration (IC_{50}) was derived [28]. To study actin assembly at pointed ends gelsolin-actin seeds (GA_2) were prepared by incubating 0.5 μM gelsolin and 1.1 μM G-actin in G buffer in the presence of excess $CaCl_2$ (2.5 mM) [25]. The assembly of G-actin (1.2 μM, containing 5 % pyrenyl-actin) from GA_2 seeds (33 nM) was monitored in the absence or presence of different amounts of SALS.

Bulk depolymerization kinetics experiments: Pre-polymerized actin filaments (1 μM, containing 50 % pyrenyl-actin) were diluted to 20 nM in the absence or presence of different amounts of SALS, gelsolin (0.1 or 0.5 μM) or Latrunculin A (20 μM). When tropomyosin was used first, actin filaments were incubated with excess tropomyosin (4.5 μM) overnight at 4°C. Kinetics of actin filament disassembly was monitored by the decrease in pyrenyl fluorescence emission as a function of time.

Critical concentration experiments: G-actin (containing 5 % pyrenyl-actin) at different concentrations were polymerized, then either SALS-WH2 (0.5 μM or 1.5 μM) or gelsolin (0.01 μM) was added and the samples were incubated overnight at 4°C. The fluorescence emission of the samples was measured and plotted as the function of the [actin] (J(c) plot). The steady-state amount of unassembled actin is reflected by the breaking point of the J(c) plot. The steady-state pool of unassembled actin is composed of free G-actin (critical concentration, c_c) and SALS-WH2-bound (sequestered) G-actin, therefore it can be related to the critical concentration, the total [SALS-WH2] and the dissociation equilibrium constant of the SALS-WH2:G-actin complex.

Steady-state experiments to determine the actin monomer:filament ratio: Actin filaments (2.5 μM, containing 5 % of pyrenyl-actin) were polymerized in the absence and presence of phalloidin (2.5 μM), jasplakinolide (2.5 μM) or tropomyosin (6 μM) overnight at 4°C. Then different amounts of SALS-WH2 were added and the samples were further incubated overnight at 4°C. The fluorescence emission of the samples was measured and the relative pyrenyl fluorescence values were plotted against the SALS-WH2 concentration.

Total internal reflection fluorescence microscopy (TIRFM) experiments

Polymerization of G-actin (0.5 μM containing 10 % Alexa®488NHS-G-actin) at the level of individual filaments was investigated by TIRFM in the absence and presence of different amounts of SALS-WH2. Actin filaments were attached to N-ethylmaleimide (NEM) labeled skeletal muscle myosin II functionalized glass surface to ensure that actin assembly can be visualized in the TIRF excitation field. The growth rates of actin filaments were determined and were plotted as the function of SALS-WH2 concentration, the quantitative analysis gave the half-inhibition concentration (IC_{50}).

High-speed sedimentation experiments

High-speed centrifugation experiments were performed to investigate the effect of SALS on monomer:filament equilibrium and the binding of SALS to the sides of actin filaments using F-actin (1, 1.5 and 2.5 μM) or phalloidin-F-actin (1 μM). The samples were ultracentrifuged (300.000 g, 30 min, 20ºC). The pellets and supernatants were separated and processed for SDS-PAGE analysis. The protein content of the pellets and the supernatants was derived. The intensity values were corrected for the molecular weight of each protein. Then, the values were converted to actin concentration ([G-actin] or [F-actin]) using a calibration curve. The slope of the linear fit to the [SALS-WH2]-[G-actin] or [SALS-WH2]-[F-actin] curves can be related to the critical concentration of actin assembly, the total [SALS-WH2] and the dissociation equilibrium constant of the SALS-WH2:G-actin complex.

RESULTS AND DISCUSSION

The WH2 domains of SALS bind actin monomers

Based on steady-state fluorescence emission and anisotropy measurements we found that SALS-WH2 binds actin monomers with sub-micromolar affinity (K_D ~ 0.23 - 0.4 μM). The binding was accompanied by marked changes in the fluorescence emission of IAEDANS attached to Cys^{374} (decrease in the fluorescence emission and redshift of the maximum wavelength). This implies that the binding of the WH2 domains affects the local milieu of the fluorophore, resulting in an altered, potentially more solvent-exposed environment in the complex. No changes were observed in the fluorescence emission of Alexa488NHS. Alexa488NHS coupled to Lys^{328} is further away from the actin-binding side of WH2 domains. We propose that Alexa488NHS is a more accurate probe to derive the WH2 domain:G-actin stoichiometry in the complexes. Importantly, the stoichiometry of SALS-WH2:G-actin complex depended on the applied fluorophore (for IAEDANS 1 : 1, for Alexa488NHS 1 : 2).

These observations support that both WH2 domains of SALS interact with one actin monomer. Also, SALS-WH2 adapts to the characteristic G-actin binding mode of WH2 domains by inserting its N-terminal amphipathic α-helix into the hydrophobic cleft between subdomain 1 and 3 of G-actin [12, 13].

The WH2 domains of SALS inhibit actin assembly

It was proposed that the full-length SALS could facilitate sarcomeric actin filament assembly at the pointed ends [1, 17]. Pyrenyl polymerization measurements revealed that the WH2 domains of SALS inhibited actin assembly in a concentration-dependent manner. The half-inhibition (IC_{50}) was reached at ~ 0.3 μM SALS-WH2. The effects of SALS-WH2 on actin polymerization at the level of individual filaments were investigated by using total internal reflection fluorescence microscopy (TIRFM). SALS-WH2 inhibited filament elongation in a concentration-dependent manner, the analysis of the data gave dissociation equilibrium constant of ~ 0.4 μM. Although, quantitative analysis was not performed but the inspection of TIRFM images suggested that SALS-WH2 reduces not only the growth rate but also the number of actin filaments.

The K_D value determined from TIRFM measurements is close to that derived from steady-state fluorescence experiments, suggesting that the ability of SALS-WH2 to inhibit polymerization arises from its monomer binding. According to these observations the WH2 domains of SALS inhibit both the nucleation and elongation phases of actin assembly. This activity of SALS-WH2 is opposite to the filament assembly promoting activity proposed for the full-length protein [17].

In the initial study on SALS it was found that the full-length protein slows down the assembly of gelsolin-capped actin filaments, which was interpreted as the association of SALS to filament pointed ends [17]. To test this, actin polymerization was induced from gelsolin-actin seeds (GA_2) in pyrenyl polymerization assays. According to our results, SALS-WH2 inhibits spontaneous actin assembly from GA_2 seeds in a similar manner to its effects on spontaneous actin polymerization. These results show that the WH2 domains of SALS are not sufficient to promote pointed end assembly.

The WH2 domains of SALS sequester actin monomers

The inhibitory effect of SALS-WH2 on actin polymerization can arise from sequestration and/or barbed end capping. The effect of SALS-WH2 on actin filament end dynamics was examined in critical concentration measurements. The breaking point appeared at c_c ~ 0.15 μM (that is characteristic of barbed end dynamics) in the absence of SALS-WH2. The addition of gelsolin to actin resulted in a shift in the critical concentration up to c_c ~ 0.6 μM that is characteristic of the pointed ends. The J(c) plots obtained in the presence of SALS-WH2 were parallel to the plot obtained for actin. The breaking point of the plots in the presence of 0.5 μM and 1.5 μM SALS-WH2 were shifted to higher values of ~ 0.3 μM and ~ 0.8 μM, respectively. Based on the quantitative analysis of our results, we reasoned that SALS-WH2 is likely to increase the amount of unpolymerized actin by sequestration and not by filament end capping.

The sequestration activity of SALS-WH2 domains was confirmed in sedimentation experiments. The steady-state amount of actin in the supernatant (monomers and/or smaller oligomers) increased and, in parallel, the steady-state amount of filamentous actin detected in the pellet decreased with increasing concentrations of SALS-WH2. At different actin concentrations, both the increase in the amount of G-actin and the decrease in the amount of F-actin followed a linear tendency and were characterized by parallel straight lines. The linear fit to the data resulted in the same absolute value of the slopes: 0.36 ± 0.02. This value matches well with the dissociation equilibrium constant of the SALS-WH2:G-actin complex and the barbed end critical concentration, supporting that SALS affects the monomer:filament equilibrium by sequestration and not by filament end capping.

The WH2 domains of SALS enhance actin filament disassembly

To address whether the WH2 domains of SALS can actively accelerate actin disassembly, dilution-induced depolymerization assays were performed. The results clearly showed that SALS-WH2 can actively enhance filament depolymerization at a rate characteristic of severing activity.

The severing activity of SALS-WH2 requires direct protein interactions with the side of actin filaments. In steady-state fluorescence spectroscopy experiments, we found that SALS-WH2 binds to phalloidin-, or jasplakinolide-stabilized actin filaments but fails to disassemble them. Using this observation, we could assay the direct interaction between the WH2 domains of SALS and the side of actin filaments quantitatively in high-speed sedimentation assays. The amount of SALS-WH2 detected in the pellet was higher (~ μM), than the one expected due to filament end binding (~ nM). This suggests that the WH2 domains of SALS interact with the side of actin filaments. The SALS-WH2:F-actin ratio in the pellet derived from these experiments reached saturation at ~ 0.5. This stoichiometry indicates that both WH2 domains of SALS bind to actin, consistent with the results obtained in steady-state fluorescence spectroscopy measurements. Based on the above results we propose that the WH2 domains of SALS can facilitate actin filament disassembly upon direct binding to the side of F-actin, so they have severing activity.

The effects of actin-binding proteins on the activities of WH2 domains

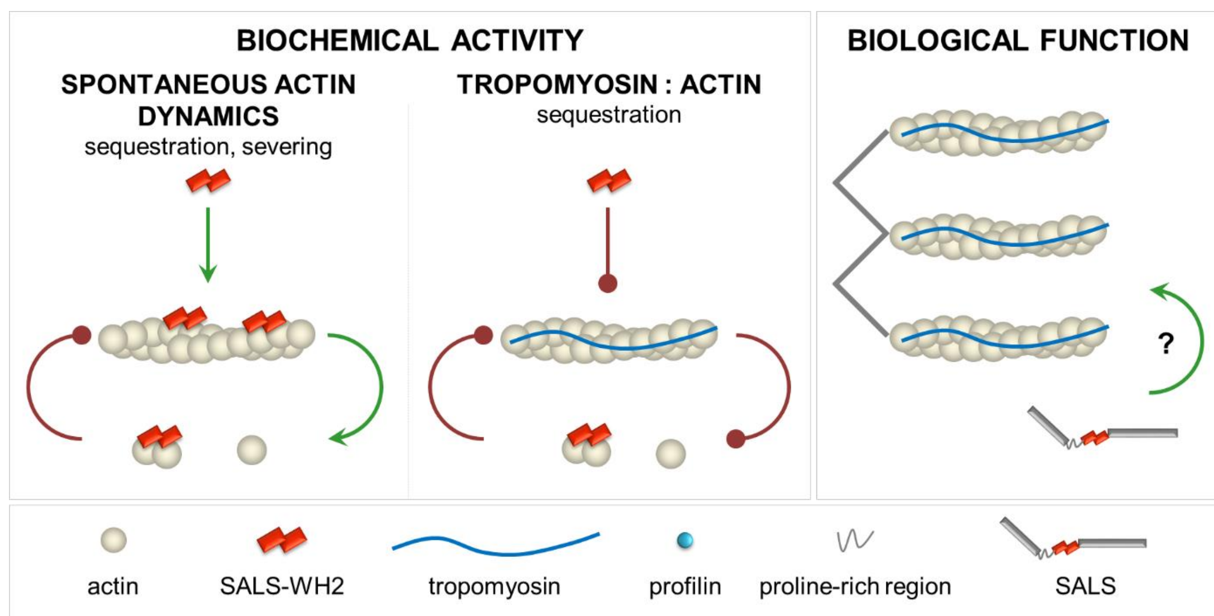
Similarly to some other WH2 domain proteins (the activators of the Arp2/3 complex, VASP, MIM, WIP, Cordon Bleu and Leiomodin [29]), SALS possesses a region rich in proline amino acids upstream to the WH2 domains. The proline-rich region is a frequent motif in actin-binding proteins, it can collaborate with profilin and this interaction can influence the effects of the actin-binding domains on actin dynamics [24, 25, 27, 30-32]. To study this issue, we investigated the biochemical activities of a construct containing both the proline-rich sequence element and the WH2 domains (SALS-Pro-WH2). Our results indicate that this region of SALS does not bind profilin and does not affect the activities of the WH2 domains of SALS either. This suggests that the proline-rich region may function as a flexible structural element in the protein.

Tropomyosin - a canonical F-actin binding element in sarcomeres - reduced the ability of SALS-WH2 to enhance the disassembly of actin filaments in dilution-induced depolymerization experiments. However, at equilibrium the amount of unassembled actin was the same both in the absence and presence of tropomyosin based on the results of steady-state pyrene fluorescence emission experiments. Tropomyosin maintains filament end dynamics and, hence it does not influence the accumulation of unassembled actin at steady-state due to sequestration [33, 34]. The high-resolution structure of the Tpm:F-actin complex reveals that the Tpm binding site overlaps with that of SALS [35]. Based on these considerations, we propose that tropomyosin solely inhibits the severing activity of SALS-WH2.

Steady-state anisotropy measurements revealed that SALS-WH2 directly interacts with tropomyosin. The dissociation equilibrium constant of SALS-WH2:Tpm complex obtained at different ionic strength conditions were as follows; SALS-WH2: $K_D = 1.1 \pm 0.2$ μM (5 mM), K_D

= 41.3 ± 7.7 μM (32 mM), K_D > 100 μM (59 mM); SALS-Pro-WH2: K_D = 0.7 ± 0.4 μM (5 mM). These results indicate the electrostatic nature of the interaction. We propose that the SALS-WH2:Tpm interaction does not rely on the WH2 domains, rather it is mediated by other sequence elements of the construct. It is worth of note that tropomyosin binding is a common feature of SALS and Leiomodin. Tpm can bind to TMBS1 binding site of Lmod at the N-terminus. It is known, that the Lmod:Tpm interaction enhances the actin nucleation activity of Lmod [8, 9].

According to my work, the activities of the WH2 domains do not reconstitute the effect of the full-length SALS protein on thin filament dynamics (*1. figure*) [1, 17]. Collectively, these observations imply that other regions in the protein and/or other sarcomeric elements must be important to modulate the biochemical activities of the WH2 domains.



1. figure Schematic representation of the biochemical activities of SALS-WH2 and the proposed biological function of the full-length SALS protein.

SUMMARY

The WH2 domains of SALS interact with both monomeric and filamentous actin. As a functional consequence of the interaction the monomer:filament equilibrium is shifted towards monomeric actin by the sequestering and severing activities of the WH2 domains.

- SALS-WH2 adapts to the characteristic G-actin binding mode of WH2 domains.
- SALS-WH2 binds actin monomers with sub-micromolar affinity.
- The stoichiometry of SALS-WH2:G-actin complex is 1:2, so both of the WH2 domains can bind one actin monomer.
- The functional consequence of the monomer binding of SALS-WH2 is the sequestering activity, through which it inhibits monomer incorporation into filaments at both ends.
- The WH2 domains of SALS interact with the side of actin filaments.
- The functional consequence of the filament binding of SALS-WH2 is the severing activity, through which it actively enhances filament depolymerization.
- The proline-rich region of SALS does not bind profilin and does not influence the activities of WH2 domains in actin dynamics, either.
- Tropomyosin inhibits the severing activity of SALS-WH2.
- SALS-WH2 binds tropomyosin, the binding is governed by electrostatic interaction.

The biochemical activities of the WH2 domains of SALS described in this work do not reconstitute the biological function of the full-length protein in sarcomeric actin dynamics. 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.

LIST OF PUBLICATIONS

Publications related to my Ph.D. work

Mónika Ágnes Tóth, Andrea Majoros, Andrea Teréz Vig, Ede Migh, Miklós Nyitrai, József Mihály, Beáta Bugyi: Biochemical Activities of the Wiskott-Aldrich Syndrome Homology Region 2 Domains of Sarcomere Length Short (SALS) Protein. *JOURNAL OF BIOLOGICAL CHEMISTRY* ***291***: pp. 667 - 680. (2016)

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Conference talks related to my Ph.D. work

1. Tóth Mónika Ágnes, Bugyi Beáta, Nyitrai Miklós, Mihály József, Migh Ede: SALS, egy WH2 domén fehérje szerepe a szarkomer aktin hálózatában. *43. Membrán - Transzport konferencia*, Sümeg, 2013. 05. 21 - 24.
2. Tóth Mónika Ágnes, Bugyi Beáta, Nyitrai Miklós, Mihály József, Migh Ede: SALS, egy WH2 domén fehérje szerepe a szarkomer aktin hálózatában. *II. Interdiszciplináris Doktorandusz Konferencia*, Pécs, 2013. 05. 15 – 17.
3. Mónika Ágnes Tóth, Beáta Bugyi, Miklós Nyitrai, József Mihály, Ede Migh: SALS, a WH2-protein in sarcomeric actin assembly. *Novel Biophysical Approaches in the Investigation of the Cytoskeleton, The 27th Europen Cytoskeletal Forum Meeting*, Pécs, 2012. 11. 3 - 7.
4. Mónika Ágnes Tóth, Beáta Bugyi: SALS, a WH2-protein in sarcomeric actin assembly. *Summer school on Actin Dynamics*, Regensburg, Germany, 2012. 09. 29 – 10. 05.
5. Tóth Mónika Ágnes, Bugyi Beáta, Nyitrai Miklós, Mihály József, Migh Ede: A SALS szerepe a szarkomer aktin hálózatának szabályozásában. *A Magyar Élettani Társaság, a Magyar Anatómusok Társasága, a Magyar Biofizikai Társaság és a Magyar Mikrocirkulációs és Vaszkuláris Biológiai Társaság Kongresszusa*, Debrecen, 2012. 06. 10 - 13.

Conference posters related to my Ph.D. work

1. Mónika Ágnes Tóth, Péter Gaszler, Judit Viktória Fórizs, Andrea Teréz Vig, Ede Migh, József Mihály, Beáta Bugyi: Biochemical activities of the Wiskott-Aldrich syndrome homology region 2 domains of Sarcomere Length Short protein. *Annual Conference of the Hungarian Biochemical Society*, Szeged, 2016. 08. 28 - 31.
2. Mónika Ágnes Tóth, Péter Gaszler, Andrea Teréz Vig, Ede Migh, József Mihály, Beáta Bugyi: Biochemical activities of the Wiskott-Aldrich syndrome homology region 2 domains of Sarcomere Length Short protein. *European Cytoskeletal Forum*, Cambridge, UK, 2016. 06. 20 - 23.

3. Tóth Mónika Ágnes, Majoros Andrea, Mihály József, Migh Ede, Nyitrai Miklós, Bugyi Beáta: SALS, egy WH2 domén fehérje szerepe a szarkomer aktin hálózatában. *44. Membrán-Transzport konferencia*, Sümeg, 2014. 05. 20 - 23.
4. Mónika Ágnes Tóth, József Mihály, Ede Migh, Lajos Kalmár, Miklos Nyitrai, Beáta Bugyi: SALS, a WH2 domain protein in sarcomeric actin organization. *ASCB Annual Meeting*, New Orleans, USA, 2013. 12.14 - 18.

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