Journal of Bioinformatics and Computational Biology
Vol. 6, No. 4 (2008) 869–884

Imperial College Press
www.icpress.co.uk

ACTIN-BINDING PROTEINS: HOW TO REVEAL THE CONFORMATIONAL CHANGES

NATALIA SINITSINA*, IGOR ORSHANSKY* and OLGA SOKOLOVA*,†,‡

*Faculty of Biology, Moscow State University, GSP-1
1 Leninskie Gory, Bld 12, 119991 Moscow, Russia

†N.A. Bakh Institute of Biochemistry, RAS
33 Leninsky Ave., Bld 2, 119071 Moscow, Russia
‡sokolova@moldyn.org

Received 16 October 2007
Revised 31 December 2007
Accepted 4 January 2008

Actin is the most abundant protein in eukaryotes. Under physiological conditions, it can polymerize into polarized filaments. At the heart of these processes are actin-binding proteins that stimulate actin assembly. Most of them are composed of multiple domains that perform both regulatory and signaling functions. Many actin-binding proteins, including WASP and formin family proteins, are auto-inhibited through intramolecular interactions that mask the actin-regulating sites of these proteins. The large flexible molecules of formins have so far eluded crystallization, and have been crystallized only partially. The information from the available crystal structures is valuable, but somewhat difficult to interpret without a larger framework on which to pose the actin-binding mechanism. Single-particle electron microscopy and electron tomography could provide such a large framework with the full-length structures of protein complexes. The recent advances in determining the molecular interactions in protein complexes predict that the molecular modeling and molecular dynamics methods could be employed to study conformational changes in molecules.

Keywords: Actin-binding proteins; domain organization; conformational changes; single-particle electron microscopy; molecular dynamics.

1. Introduction

Actin is the most abundant protein in eukaryotes. Under physiological conditions, it can polymerize into polarized filaments, characterized by a fast-growing (barbed) end and a slow-growing (pointed) end.[1,2] Cell locomotion, endocytosis, and intracellular motility of vesicles, organelles, and pathogens all rely on the rapid assembly of actin networks. Some deathly diseases, such as anthrax[3] and Alzheimer's disease,[4] affect the actin-based cell motility. In yeast (*S. cerevisiae*), directional reorganization of the actin cytoskeleton allows the polarized cell growth that is necessary for bud emergence.[5,6]

At the heart of these processes are actin-binding proteins (ABPs) that, upon activation by various factors such as SCAR/WASP family proteins, stimulate actin assembly (formins, Arp2/3 complex) and disassembly (gelsolin, ADF/cofilin, AIP1).[7] Formins are thought to processively cap the fast-growing ends of actin filaments,[6] while the Arp2/3 complex seeds actin polymerization by forming a pseudo-actin trimer of its two actin-related subunits Arp2 and Arp3 bound to WASP.[8,9] Arp2/3 complex activation stimulates the formation of membrane protrusions downstream of the Rho-family GTPases. Recent studies demonstrated that the formation of membrane protrusions also depends on controlled interplay between direct membrane deformation by IRSp53/MIM family proteins and the actin cytoskeleton.[10]

Most ABPs are composed of multiple domains, performing both regulatory and signaling functions. Many of them, including WASP and formin family proteins, are auto-inhibited through intramolecular interactions that mask the actin-regulating sites of these proteins.[11,12]

The X-ray structures of various small ABPs were recently discovered,[13–18] outlining some valuable functional information. Yet large oligomeric complexes such as formins and CAP/srv2 have been crystallized only partially,[15–20] and therefore the precise mechanism by which they control the assembly and turnover of actin cytoskeleton remains unclear. The size and flexibility of such modules predict that they will not be amenable to the traditional structural techniques of nuclear magnetic resonance (NMR) and X-ray crystallography; NMR is restricted to molecules of usually less than 40 kD. On the other hand, many actin-binding proteins assemble into high-molecular-weight complexes (for example, yeast formin Bni1 accomplishes several megadaltons). Indeed, X-ray crystal structures have been determined for only two fragments of the large formin molecule: the N-terminal domain[20] and the C-terminal "donut."[15,18]

In this review, we will focus on the domain organization of key ABPs (e.g. formins, Arp2/3 complex activators, and IMD-related proteins) and the recent advances in revealing their structure and conformational changes.

2. Domain Organization of ABPs

2.1. *Formins*

Formins are modular proteins containing a series of domains and functional motifs [Fig. 1(a)]. Metazoan formins fall into seven groups: Dia (diaphanous), Daam (disheveled-associated activator of morphogenesis), FRL (formin-related gene in leukocytes), FHOD (formin homology domain-containing protein), INF (inverted formin), FMN (formin), and delphilin.[21] The oligomeric state of formins was determined by means of analytical ultracentrifugation. A strong correlation was detected between the ability of formins to form stable dimers and the ability to promote actin assembly, suggesting a dimer as the functional state of formins.[22] Each monomer is comprised of the highly conserved formin homology 2 (FH2) domain and its

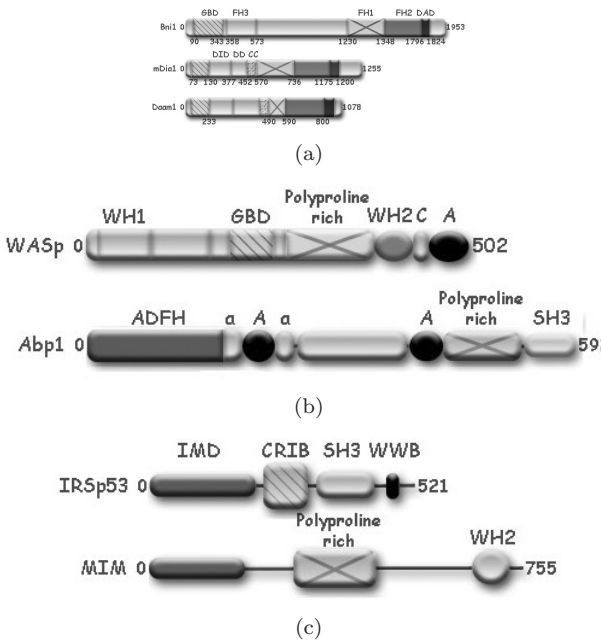

Fig. 1. Schematic representation of the domain organization of actin-binding proteins (ABPs). (a) Comparative domain structure of formins; (b) domain structure of Arp2/3 complex activators; and (c) domain composition of ABP containing IMD domains. Designation of domains: GBD/CRIB – Rho GTPase binding domain; DID – diaphanous inhibitory domain; DD – dimerization domain; CC – coiled coil; FH – formin homology domain; DAD – diaphanous auto-regulatory domain; WH – WASP homology domain; IMD – IRSp53/MIM homology domain; C – central domain; WHD – WAVE-homology domain; A – acidic domain; SH3 – Src homology 3 domain; WWB – WW domain-binding motif.

neighboring proline-rich formin homology 1 (FH1) domain, which was predicted to be unstructured,[11] surrounded by regulatory domains: N-terminal GTPase binding domain (GBD), an FH3 region that may be important for localization,[23] and the DID and DAD domains.

The defining features of formin proteins are their FH domains. FH1 domains are highly variable in length (could vary from 15 to 229 residues), proline content (35%–100%), and number of profilin-binding sites (0–16).[24,25] Profilin can bind to an actin monomer and a polyproline sequence of FH1 simultaneously, and both interactions are in rapid equilibrium.[26]

The FH2 domain is a $\sim$400-amino-acid sequence that is crucial for its effects on actin nucleation and elongation of new actin filaments. Two crystal structures of the FH2 domains are available to date: one from the budding yeast formin Bni1,[15] and one from the disheveled-associated activator of morphogenesis (Daam1).[18] Both structures reveal the dimeric FH2 domain, with the two subunits surrounding a large central "donut hole". The unique mechanism of actin filament nucleation by FH2 domains will be discussed later in this paper.

2.2. *Arp2/3 complex activators*

The Arp2/3 complex promotes the branching of the filament by providing the platform for nucleation at its side, while forming a pseudo-actin trimer of the two actin-related subunits, Arp2 and Arp3, bound to WASP.[9] The nucleation is activated by SCAR/WASP proteins.[9,27] The mechanism of activation involves stabilizing the Arp2/3 complex in a closed conformation (see Fig. 3).[9] The domain organization of WASP is depicted in [Fig. 1(b)]. The C-terminal regions of WASP family proteins contain one or two copies of the actin-monomer binding domain WASP homology 2 domain (WH2), which is important for the actin-filament nucleating activity of the WASP–Arp2/3 complex,[28,29] followed by an acidic domain that binds to and activates the actin-filament nucleator Arp2/3.[30,31] The WH2 domain, the cofilin-homology domain (C), and the acidic domain (A) comprise the so-called VCA region [Fig. 1(b)].

The related Arp2/3 complex activator, WASP family verprolin homologous protein (WAVE; called SCAR in *Dictyostelium*), shares the C-terminal domains, the VCA region, with WASP, but possesses a different N-terminal domain (WHD), predicted to be a coiled-coil region and to form the pentameric heterocomplex that is necessary for localization and stability of WAVE.[32] WAVE proteins also lack the GBD domain. Therefore, they do not directly bind small GTPases, but adopt an indirect mechanism of activation through interacting with the SH3-domain ABPs bound to Rac.

A recently identified Arp2/3 activator, Abp1, is a highly conserved protein that was first found in *S.cerevisiae*[33] and was thought to link functions of the actin cytoskeleton to endocytosis.[34,35] Abp1 is a multi-domain protein consisting of an N-terminal actin depolymerizing factor homology (ADFH) domain required for actin filament binding,[5,36] two centrally located acidic motifs, a proline-rich region, and a C-terminal SH3 domain [Fig. 1(b)].[37] Abp1 binds to actin filaments, but does not affect their dynamics.[5,36] It has been shown that Abp1 activates the Arp2/3 complex, apparently by forming a direct association with the Arp2/3 complex and stimulating its actin nucleation activity.[5] Abp1 and its mammalian relative cortactin have been categorized as class II nucleation promoter factors because they bind to F-actin, but not G-actin.[38] The crystal structure of the Abp1 ADFH domain is very similar to that of ADF/cofilin[39] and twinfilins, yet different proteins have highly distinct effects on actin dynamics.[37,40,41] Whereas twinfilins have an affinity for actin monomers,[42,43] ADF/cofilins and Abp1 interact with F-actin. ADF/cofilins promote filament severing and depolymerization, whereas Abp1 links F-actin and the Arp2/3 complex[39] to promote nucleation of actin filaments upon activation by SH3 domain ligands.[44]

2.3. *ABPs containing IM domains*

Two novel ABPs — MIM (Missing In Metastasis)[45] and IRSp53 (insulin receptor tyrosine kinase substrate p53) — are large multi-domain proteins that regulate actin

dynamics and the motility of animal cells.[46,47] Both proteins are members of a new family of actin cytoskeleton adaptor proteins. They possess a novel mechanism that links actin filaments and the plasma membrane. Both proteins interact with actin through their C-terminal WH2 domain, while promoting the plasma membrane deformation through their N-terminal IM domain.[47] Their C-terminal SH3 domain also provides a molecular link between small GTPases and WAVE2 [Fig. 1(c)].

Structural work demonstrated that the IM domain is a "zeppelin-shaped" homodimer.[45,48] Interestingly, this domain shows structural similarity to the BAR domain, a membrane-deforming domain involved in the generation of plasma membrane invaginations.[49] The membrane-binding interfaces of IM and BAR domains display opposite curvatures and tubulate membranes to opposite directions.[47]

3. Proposed Conformational Changes in ABPs

3.1. *Conformational changes during activation*

It is hypothesized that the N-terminal DID domain inhibits an actin assembly by the C-terminal domain in formins [Fig. 2(a)].[28] Deletion of the DAD domain results in a 20,000-fold decrease in inhibitory potency for the truncated constructs *in vitro*.[29] Sequence alignments show that three metazoan formin groups possess clear DID sequences: Dia, FRL, and Daam.[21] Members of these groups also contain

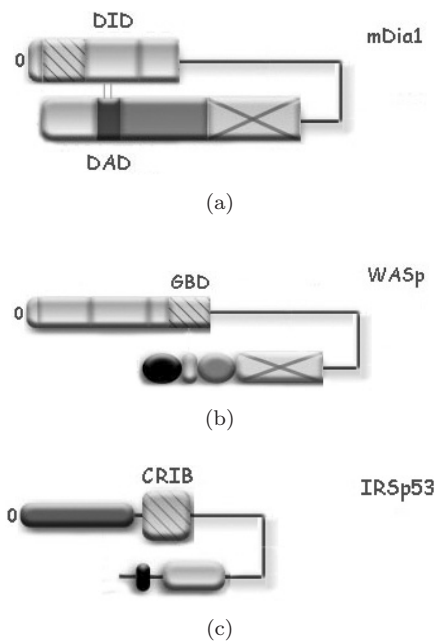

Fig. 2. Proposed conformational changes in auto-inhibited ABP molecules. (a) mDia1; (b) WASP; and (c) hypothetical conformation of auto-inhibited IRSp53.

DAD regions. Thus, Dia, FRL, and Daam formins are likely to be auto-inhibited too. The yeast formin Bni1 lacks the DID sequence and it was shown to be active. Nezami *et al.*[50] cocrystallized the DID domain (residues 131–369 of murine Dia1) with the DAD segment (residues 1,175–1,200). They revealed that the bound DAD peptide makes an extensive hydrophobic contact with the DID domain through an amphipathic helix, suggesting direct DID–DAD interaction during auto-inhibition.

The auto-inhibition of formins is revealed by small Rho GTPases (listed in Table 1). The specific Rho binds to the N-terminal GBD domain, partially overlapping with the DID domain. RhoA competes with DAD for binding the N-terminus of mDia1.[28] The precise mechanism of the RhoA and DAD competing is poorly understood. Although active RhoA is able to completely displace DAD, bound to the isolated mDia1 N-terminus in a competition assay,[19] Rho appears to only partially relieve DAD-mediated auto-inhibition of actin assembly *in vitro*.[29] This observation suggests the possibility that some other proteins or mechanisms might also be involved in formin activation.

The N-terminus of mammalian WASP also possesses an auto-inhibitory function. The auto-inhibited molecule is partially folded, thus preventing an association of its C-terminus with the Arp2/3 complex [Fig. 2(b)]. Upon binding to stimulatory molecules, such as Cdc42, mammalian WASP unfolds, exposing its Arp2/3 complex activation domain.[51,52] In contrast, WAVE is constitutively active, similar to the yeast WASP homolog Las17.[53]

Recent studies revealed that both MIM and IRSp53 exist *in vivo* in an auto-inhibited conformation.[46,48] The possible conformational change of IRSp53 is depicted in Fig. 2(c). In the case of IRSp53, the auto-inhibition is released through

Table 1. Actin-binding proteins and their binding partners.

Proteins	Conformation *in vivo*	Ligand	Where to bind	Result	Reference
Formins					
mDia1	Auto-inhibited dimer	RhoA Bud6	DID/GBD DAD	Activation	19, 20, 29 22
Bni1	Active dimer	Bud6	FH2/DAD	Stimulation	69
Arp2/3 complex activators					
WASP	Auto-inhibited monomer	Cdc42	GBD	Activation	70
WAVE	Active heterocomplex	IRSp53/Rac	PP	Activation	74
IMD containing					
IRSp53	Auto-inhibited dimer	Cdc42 Rac1	CRIB IMD	Activation	71 72
MIM	Auto-inhibited dimer	Rac	IMD	Activation	73

an interaction with small GTPases Rac1 and Cdc42 (Cdc42 binds to the CRIB motif, while Rac1 binds to a specific sequence within the IMD domain). The association of Rac1 or Cdc42 is proposed to liberate the C-terminal SH3 domain, thereby allowing the SH3 domain to interact with its binding partners. The activators of MIM have not been identified yet.[54]

Generally, the major redistribution of domains should be easily detectable using direct methods such as X-ray crystallography and electron microscopy (EM), yet such major movement makes the molecule too flexible and not readily to form diffraction quality crystals for X-ray studies. An example of such a flexible molecule is the yeast formin Bni1. A single-particle EM analysis of negative-stained Bni1 protein revealed that the molecule is almost 100 nm long and possesses an "open" conformation (Sokolova, unpublished results). This is consistent with the finding that Bni1 is active *in vivo*.

3.2. *Conformational changes in the process of actin binding*

An important property of the reviewed ABPs is to regulate the actin dynamics. Formins directly nucleate actin polymerization by a novel mechanism, recently reviewed by Goode and Eck.[55] It was hypothesized that the dimer of FH2 domains that forms a "donut"[15,18] moves processively with an elongating actin filament barbed end, thus preventing association of conventional capping proteins with the filament.

There are several models for FH2 domain processivity.[15,22,27,56,57] It is predicted that each subunit of the FH2 dimer binds one actin subunit at the barbed end, and that the FH2 dimer "stair-steps" with the elongating filament. One model predicts that only one FH2 subunit is bound at a time, with the other site free to accept a new monomer on the filament[22]; according to this model, the bound FH2 subunit interacts directly with the barbed end. Another model suggests that both FH2 subunits might bind simultaneously to the sides of the two barbed-end subunits, and that the addition of a monomer to the filament causes one FH2 subunit to release its previous actin and then bind to the newly added actin.[27] A third model suggests that interior residues of the FH2 "donut" domain interact with the barbed end, and that anticooperative binding of the FH2 and the actin subunits enables processive movement.[15] All models predict that the formin molecule undergoes a large conformational change upon such "stair-stepping".

The activity of MIM and IRSp53 is linked to the actin filament assembly and formation of plasma membrane protrusions. The N-terminal IMD domains of both proteins interact with the inner surface of the plasma membrane, stimulating direct membrane deformation. It was proposed that the IMD domain undergoes a conformational change, large enough to induce the formation of a membrane tubule with a diameter of approximately 95 nm.[47] Additionally, the C-terminal WH2 domain may move away to cross-link F-actin to the inner surface of the filopodia.

4. Methods Used to Reveal the Conformational Changes

The structure of ABPs that regulate the actin dynamics has been studied extensively in the past decade. A number of crystal structures of the whole proteins and fragments of the larger molecules have arisen, outlining valuable functional information.[13–18] Unfortunately, the large domain motion has yet to be directly observed at molecular resolution. All hypotheses describing the conformational changes have come solely from indirect data, such as crystallographic studies and molecular modeling. The few cases when the conformational change was directly observed within ABPs will be discussed below.

4.1. *Indirect methods*

Indirect methods build up the platform.

X-ray crystallography provides the molecular structures of individual small proteins and their domains.[13–18] The basic limitation of this method is that it usually provides only one conformation of the single protein, while most of the ABPs exist in equilibrium of different conformations. For example, the Arp2/3 complex was crystallized in inactive conformation, with both actin-related subunits, Arp2 and Arp3, situated far from each other.[14] The authors concluded that the Arp2/3 complex should undergo a large conformational change upon activation, which was later confirmed by single-particle EM[9] and electron tomography.[58] It should be mentioned also that the interpretation of the crystal structure of the formin FH2 domain[15,22] gave rise to the hypotheses of its processive movement upon nucleating of the actin cables.[27,56,57]

The use of theoretical statistical methods for describing the kinetics of molecular interactions and determining the molecular mechanisms and conformational changes in molecules is also highly favorable.[59,60] The crystal structures of proteins or small complexes of interest can be easily downloaded from the Protein Data Bank (PDB). Recently, molecular dynamics (MD) methods have been used to investigate the processes on the interdomain interfaces, the stability and dynamical behavior, and the single amino acids in the formation of multiple protein complexes. For example, Bindshadler *et al.*[61] created a mathematical model of steady-state interaction between G- and F-actin. This model may be used for predicting the geometry and dynamics of binding of ADF/cofilins and the Arp2/3 complex. The molecular mechanisms of actin turnover have been studied intensively. Wriggers and Schulten[62] investigated the ATP hydrolysis that accompanies actin polymerization. They demonstrated that the release of cleaved phosphate proceeds through a "back door" mechanism: ATP enters and ADP leaves the actin from one side, while Pi leaves from the opposite side. Wriggers *et al.*[63] predicted the formation of a complex of yeast cofilin with G-actin by MD simulation. The new structural model revealed a possible mechanism of actin depolymerization by members of the cofilin family. Kozlov and Bershadsky[56] applied the pulling forces to actin filaments; the authors predicted the force-driven polymerization of

actin filaments based on the phenomenon of leaky capping of actin filaments by formins.

Results of MD experiments may be helpful for predicting the structure of protein–protein complexes, their stability, and the force of interdomain interaction. They can also be used to model the significance of point mutations.

4.2. *Direct methods*

The first direct evidence of processive attachment of the dimer of FH1-FH2 domains to the growing barbed end of the actin filament came from total internal reflection fluorescence microscopy (TIRFM) images. The dimer of FH1-FH2 domains of mDia1, fluorescently labeled and immobilized on the microscopy slide, remained continually associated with the barbed end of the growing filament[32]; the addition of profilin increased the barbed end elongation rate.[64]

Electron microscopy could be used successfully to provide a low-resolution structure (below 2.5 nm) of the full-length protein in its native oligomeric state. This method can distinguish among distinct conformations of the protein that fall into separate classes. Single-particle EM has several advantages. It is not limited by the size of the particle, and in fact is suited to studies of larger protein complexes. Much less material is required, especially when observed under negative stain techniques used to increase contrast of the particles. Strong contrast is critical for successful alignment of the particles.

The advantage of cryo-EM studies of unstained particles frozen in vitreous ice is that the images of particles over the holes are of higher quality, since the carbon layer adds to the noise. Cryo-EM will preserve structural detail at higher resolution and reveal the interior structure of the protein.

The single-particle EM method was employed to calculate the domain rearrangements within the yeast and bovine Arp2/3 complexes upon their activation.[9] The image processing resulted in determining three separate conformations (open, closed, and intermediate) that are depicted in Fig. 3. "Closing" of the Arp2/3 complex during activation and binding of WASP has been clearly demonstrated.

Fig. 3. Conformational changes upon activation of the Arp2/3 complex (modified from Rodal *et al.*[9]). (a) Open conformation; (b) intermediate conformation. Arrows are pointing towards the direction of domain movement. (c) Closed conformation.

Another possibility to successfully achieve the initial structural data is metal shadowing. This method was successfully used to visualize the huge oligomeric Srv2 complex, purified from yeast.[65]

4.3. *Current problems with the EM method*

Negative staining conceals the internal structure of the protein and may result in structural artifacts. For example, interactions between protein and the charged surface of the substrate (carbon film) may distort the protein conformation. Also, the increased concentration of salts and changing of the pH may result in collapse of the protein.

When the mass of the object embedded in vitreous ice is below 500 kDa, which includes most individual protein molecules or small complexes, the beam damage may be substantial. Also, a higher protein concentration is necessary to obtain a good density of particles in the holes. Because the densities of ice and protein are quite similar, images of vitrified specimens show very little contrast. The three-dimensional (3D) structure of the Arp2/3 complex, frozen in vitreous ice,[66] suffers from the weak contrast. As a result, the overall shape of the structure differs dramatically from the known crystal structure.[14] Interestingly, the projection structure of the Arp2/3 complex, embedded in negative stain, resembles very close this crystal structure, apparently due to the increased contrast of the images.[9]

The identification of domains plays an important part in the interpretation of low-resolution 3D structures. To identify the domain arrangement, crystal structures of known parts or ligands can be docked into the lower-resolution EM structure of a single protein with the molecular interaction data as a guide.

Immunolabeling is another most commonly used technique. A problem with this method is the flexibility of the full-length antibody, which makes the identification of a specific binding site difficult. To solve this problem, shorter F(ab)′ fragments are used. Additionally, the occupancy of the binding sites is far from 100%.

To visualize the bound antibodies, gold labeling may be used. However, this technique also suffers from localization problems. The gold clusters are usually difficult to bring into correlation with their binding sites. The recent discovery of a clonable metallothionein tag[67] that initiates the formation of a heavy metal cluster at the labeling site can solve these problems.

4.4. *Electron tomography*

Electron tomography has emerged as the leading method for the study of 3D ultrastructure in the 5–20-nm resolution range. It is ideally suited for studying subcellular assemblies and macromolecular complexes by offering sufficient resolution to locate the macromolecular complexes in their cellular context. Several most recent examples of the successful use of electron tomography to study actin and ABPs include the fine organization of actin networks within the intact filopodia of

Dictyostelium[68] and the visualization of an actin filament branch junction formed by the amoeba Arp2/3 complex.[58]

5. Conclusions and Perspectives

The ability to map biochemical and genetic information on a 3D model provides for deeper understanding of the function of molecules and serves as an important guide for further biochemical, genetic, and structural studies. This has been demonstrated by the large number of protein structures solved by X-ray crystallography, which provides a detailed understanding of protein folds, enzymatic catalysis, and intermolecular contacts. Nevertheless, large flexible molecules of ABPs have so far eluded crystallization, or have been crystallized only partially. The information from the crystal structures is valuable, but somewhat difficult to interpret without a larger framework on which to pose the actin-binding mechanism.

Single-particle EM can provide such a large framework with the full-length structures of protein complexes. With such a structure as the base, current and future crystal structures can be docked into the lower-resolution EM structure with molecular interaction data as a guide. The small number of EM reconstructions published to date could be explained by the difficulties in keeping the flexible molecules under live conditions to prevent degradation. The ABP molecules in auto-inhibited conformation (such as formins, MIM, and IRSp53) are therefore most favorable to work with. The application of statistical methods and molecular dynamics to study the small ABPs is also desirable, and may be useful for predicting the geometry and dynamics of conformational changes.

Acknowledgment

This work was supported in part by a grant from the presidium of the RAS "Fundamental Sciences towards Medicine" Program.

References

1. Pollard TD, Blanchoin L, Mullins RD, Molecular mechanisms controlling actin filament dynamics in nonmuscle cells, *Annu Rev Biophys Biomol Struct* **29**:545–576, 2000.
2. Disanza A, Mantoani S, Hertzog M, Gerboth S, Frittoli E, Steffen A, Berhoerster K, Kreienkamp HJ, Milanesi F, Di Fiore PP, Ciliberto A, Stradal TE, Scita G, Regulation of cell shape by Cdc42 is mediated by the synergic actin-bundling activity of the Eps8–IRSp53 complex, *Nat Cell Biol* **8**(12):1337–1347, 2006.
3. During RL, Li W, Hao B, Koenig JM, Stephens DS, Quinn CP, Southwick FS, Anthrax lethal toxin paralyzes neutrophil actin-based motility, *J Infect Dis* **192**(5):837–845, 2005.
4. Sabo SL, Ikin AF, Buxbaum JD, Greengard P, The Alzheimer amyloid precursor protein (APP) and FE65, an APP-binding protein, regulate cell movement, *J Cell Biol* **153**(7):1403–1414, 2001.
5. Goode BL, Rodal AA, Modular complexes that regulate actin assembly in budding yeast, *Curr Opin Microbiol* **4**(6):703–712, 2001.

6. Sagot I, Rodal AA, Moseley J, Goode BL, Pellman D, An actin nucleation mechanism mediated by Bni1 and profilin, *Nat Cell Biol* **4**:626–631, 2002.
7. Ono S, Mechanism of depolymerization and severing of actin filaments and its significance in cytoskeletal dynamics, *Int Rev Cytol* **258**:1–82, 2007.
8. Pollard TD, Regulation of actin filament assembly by Arp2/3 complex and formins, *Annu Rev Biophys Biomol Struct* **36**:451–477, 2007.
9. Rodal AA, Sokolova O, Robins DB, Daugherty KM, Hippenmeyer S, Riezman H, Grigorieff N, Goode BL, Conformational changes in the Arp2/3 complex leading to actin nucleation, *Nat Struct Mol Biol* **12**(1):26–31, 2005.
10. Mattila PK, Pykäläinen A, Saarikangas J, Paavilainen VO, Vihinen H, Jokitalo E, Lappalainen P, Missing-in-metastasis and IRSp53 deform PI(4,5)P2-rich membranes by an inverse BAR domain-like mechanism, *J Cell Biol* **176**(7):953–964, 2007.
11. Higgs HN, Formin proteins: A domain-based approach, *Trends Biochem Sci* **30**(6):342–353, 2005.
12. Welch MD, Mullins RD, Cellular control of actin nucleation, *Annu Rev Cell Dev Biol* **18**:247–288, 2002.
13. Appleton BA, Wu P, Wiesmann C, The crystal structure of murine coronin-1: A regulator of actin cytoskeletal dynamics in lymphocytes, *Structure* **14**(1):87–96, 2006.
14. Robinson RC, Turbedsky K, Kaiser DA, Marchand JB, Higgs HN, Choe S, Pollard TD, Crystal structure of Arp2/3 complex, *Science* **294**(5547):1679–1684, 2001.
15. Xu Y, Moseley JB, Sagot I, Poy F, Pellman D, Goode BL, Eck MJ, Crystal structures of a formin homology-2 domain reveal a tethered dimer architecture, *Cell* **116**(5):711–723, 2004.
16. Dodatko T, Fedorov AA, Grynberg M, Patskovsky Y, Rozwarski DA, Jaroszewski L, Aronoff-Spencer E, Kondraskina E, Irving T, Godzik A, Almo SC, Crystal structure of the actin binding domain of the cyclase-associated protein, *Biochemistry* **43**(33):10628–10641, 2004.
17. Ksiazek D, Brandstetter H, Israel L, Bourenkov GP, Katchalova G, Janssen KP, Bartunik HD, Noegel AA, Schleicher M, Holak TA, Structure of the N-terminal domain of the adenylyl cyclase-associated protein (CAP) from *Dictyostelium discoideum, Structure* **11**(9):1171–1178, 2003.
18. Lu J, Meng W, Poy F, Maiti S, Goode BL, Eck MJ, Structure of the FH2 domain of Daam1: Implications for formin regulation of actin assembly, *J Mol Biol* **369**(5):1258–1269, 2007.
19. Rose R, Weyand M, Lammers M, Ishizaki T, Ahmadian MR, Wittinghofer A, Structural and mechanistic insights into the interaction between Rho and mammalian Dia, *Nature* **435**(7041):513–518, 2005.
20. Otomo T, Otomo C, Tomchick DR, Machius M, Rosen MK, Structural basis of Rho GTPase-mediated activation of the formin mDia1, *Mol Cell* **18**(3):273–281, 2005.
21. Higgs HN, Peterson KJ, Phylogenetic analysis of the formin homology 2 (FH2) domain, *Mol Biol Cell* **16**(1):1–13, 2005.
22. Moseley JB, Sagot I, Manning AL, Xu Y, Eck MJ, Pellman D, Goode BL, A conserved mechanism for Bni1- and mDia1-induced actin assembly and dual regulation of Bni1 by Bud6 and profilin, *Mol Biol Cell* **15**(2):896–907, 2004.
23. Petersen J, Nielsen O, Egel R, Hagan IM, FH3, a domain found in formins, targets the fission yeast formin Fus1 to the projection tip during conjugation, *J Cell Biol* **141**(5):1217–1228, 1998.
24. Pring M, Evangelista M, Boone C, Yang C, Zigmond SH, Mechanisms of formin-induced nucleation of actin filaments, *Biochemistry* **42**(2):486–496, 2003.

25. Kovar DR, Kuhn JR, Tichy AL, Pollard TD, The fission yeast cytokinesis formin Cdc12p is a barbed end actin filament capping protein gated by profilin, *J Cell Biol* **161**(5):875–887, 2003.
26. Wallar BJ, Alberts AS, The formins: Active scaffolds that remodel the cytoskeleton, *Trends Cell Biol* **13**(8):435–446, 2003.
27. Machesky LM, Mullins RD, Higgs HN, Kaiser DA, Blanchoin L, May RC, Hall ME, Pollard TD, Scar, a WASp-related protein, activates nucleation of actin filaments by the Arp2/3 complex, *Proc Natl Acad Sci USA* **96**(7):3739–3744, 1999.
28. Boldogh IR, Yang HC, Nowakowski WD, Karmon SL, Hays LG, Yates JR, Pon LA, Arp2/3 complex and actin dynamics are required for actin-based mitochondrial motility in yeast, *Proc Natl Acad Sci USA* **98**:3162–3167, 2001.
29. Madania A, Dumoulin P, Grava S, Kitamoto H, Scharer-Brodbeck C, Soulard A, Moreau V, Winsor B, The *Saccharomyces cerevisiae* homologue of human Wiskott–Aldrich syndrome protein Las17p interacts with the Arp2/3 complex, *Mol Biol Cell* **10**:3521–3538, 1999.
30. Winter D, Podtelejnikov AV, Mann M, Li R, The complex containing actin-related proteins Arp2 and Arp3 is required for the motility and integrity of yeast actin patches, *Curr Biol* **138**:375–384, 1997.
31. Pelham RJ Jr, Chang F, Role of actin polymerization and actin cables in actin-patch movement in *Schizosaccharomyces pombe*, *Nat Cell Biol* **3**:235–244, 2001.
32. Higashida C, Miyoshi T, Fujita A, Oceguera-Yanez F, Monypenny J, Andou Y, Narumiya S, Watanabe N, Actin polymerization-driven molecular movement of mDia1 in living cells, *Science* **303**:2007–2010, 2004.
33. Drubin DG, Miller KG, Botstein D, Yeast actin-binding proteins: Evidence for a role in morphogenesis, *J Cell Biol* **107**:2551–2561, 1988.
34. Qualmann B, Kessels MM, Endocytosis and the cytoskeleton, *Int Rev Cytol* **220**:93–144, 2002.
35. Engqvist-Goldstein AE, Drubin DG, Actin assembly and endocytosis: From yeast to mammals, *Annu Rev Cell Dev Biol* **19**:287–332, 2003.
36. Kessels MM, Engqvist-Goldstein AE, Drubin DG, Association of mouse actin-binding protein 1 (mAbp1/SH3P7), an Src kinase target, with dynamic regions of the cortical actin cytoskeleton in response to Rac1 activation, *Mol Biol Cell* **11**(1):393–412, 2000.
37. Lappalainen P, Kessels MM, Cope MJ, Drubin DG, *Mol Biol Cell* **9**:1951–1959, 1998.
38. Welch MD, Mullins RD, Cellular control of actin nucleation, *Annu Rev Cell Dev Biol* **18**:247–288, 2002.
39. Quintero-Monzon O, Rodal AA, Strokopytov B, Almo SC, Goode BL, Structural and functional dissection of the Abp1 ADFH actin-binding domain reveals versatile *in vivo* adapter functions, *Mol Biol Cell* **16**(7):3128–3319, 2005.
40. Carlier MF, Actin: Protein structure and filament dynamics, *J Biol Chem* **266**(1):1–4, 1991.
41. Blanchoin L, Pollard TD, Mechanism of interaction of *Acanthamoeba* actophorin (ADF/Cofilin) with actin filaments, *J Biol Chem* **274**(22):15538–15546, 1999.
42. Goode BL, Drubin DG, Lappalainen P, Regulation of the cortical actin cytoskeleton in budding yeast by twinfilin, a ubiquitous actin monomer-sequestering protein, *J Cell Biol* **142**(3):723–733, 1998.
43. Vartiainen M, Ojala PJ, Auvinen P, Peranen J, Lappalainen P, Mouse A6/twinfilin is an actin monomer-binding protein that localizes to the regions of rapid actin dynamics, *Mol Cell Biol* **20**(5):1772–1783, 2000.

44. Lila T, Drubin DG, Evidence for physical and functional interactions among two *Saccharomyces cerevisiae* SH3 domain proteins, an adenylyl cyclase-associated protein and the actin cytoskeleton, *Mol Biol Cell* **8**(2):367–385, 1997.
45. Lee SH, Kerff F, Chereau D, Ferron F, Klug A, Dominguez R, Structural basis for the actin-binding function of missing-in-metastasis, *Structure* **15**(2):145–155, 2007.
46. Yamagishi A, Masuda M, Ohki T, Onishi H, Mochizuki N, A novel actin bundling/filopodium forming domain conserved in insulin receptor tyrosine kinase substrate p53 and missing in metastasis protein, *J Biol Chem* **279**(15):14929–14936, 2004.
47. Mattila PK, Pykalainen A, Saarikangas J, Paavilainen VO, Vihinen H, Jokitalo E, Lappalainen P, Missing-in-metastasis and IRSp53 deform PI(4,5)P2-rich membranes by an inverse BAR domain-like mechanism, *J Cell Biol* **176**(7):953–964, 2007.
48. Millard TH, Bompard G, Heung MY, Dafforn TR, Scott DJ, Machesky LM, Fütterer K, Structural basis of filopodia formation induced by the IRSp53/MIM homology domain of human IRSp53, *EMBO J* **24**(2):240–250, 2005.
49. Peter BJ, Kent HM, Mills IG, Vallis Y, Butler PJ, Evans PR, McMahon HT, BAR domains as sensors of membrane curvature: The amphiphysin BAR structure, *Science* **303**(5657):495–499, 2004.
50. Nezami AG, Poy F, Eck MJ, Structure of the autoinhibitory switch in formin mDia1, *Structure* **14**(2):257–263, 2006.
51. Winter D, Lechter T, Li R, Activation of the yeast Arp2/3 complex by Bee1p, a WASP-family protein, *Curr Biol* **9**:501–504, 1999.
52. Rohatgi R, Ma L, Miki L, Lopez M, Lirchhausen T, Takenawa T, Kirschner M, The interaction between N-WASP and the Arp2/3 complex links Cdc42-dependent signals to actin assembly, *Cell* **97**:221–231, 1999.
53. Rodal AA, Kozubowski L, Goode BL, Drubin DG, Hartwig JH, Actin and septin ultrastructures at the budding yeast cell cortex, *Mol Biol Cell* **16**(1):372–384, 2005.
54. Disanza A, Steffen A, Hertzog M, Frittoli E, Rottner K, Scita G, Actin polymerization machinery: The finish line of signaling networks, the starting point of cellular movement, *Cell Mol Life Sci* **62**(9):955–970, 2005.
55. Goode BL, Eck MJ, Mechanism and function of formins in the control of actin assembly, *Annu Rev Biochem* **76**:593–627, 2007.
56. Kozlov MM, Bershadsky AD, Processive capping by formin suggests a force driven mechanism of actin polymerization, *J Cell Biol* **167**:1011–1017, 2004.
57. Otomo T, Tomchick DR, Otomo C, Panchal SC, Machius M, Rosen MK, Structural basis of actin filament nucleation and processive capping by a formin homology 2 domain, *Nature* **433**:488–494, 2005.
58. Rouiller I, Xu XP, Amann KJ, Egile C, Nicastro D, Li R, Polland TD, Volkmam N, Hanein D, The structural basis of actin filament branching by Arp2/3 complex, *J Cell Biol* **180**:887–895, 2008.
59. Cavalli A, Prota AE, Stehle T, Dermody TS, Recanatini M, Folkers G, Scapozza L, A molecular dynamics study of reovirus attachment protein σ1 reveals conformational changes in σ1 structure, *Biophys J* **86**(6):3423–3431, 2004.
60. Mendieta J, Ramírez G, Gago F, Molecular dynamics simulations of the conformational changes of the glutamate receptor ligand-binding core in the presence of glutamate and kainite, *Proteins* **44**(4):460–469, 2001.
61. Bindschadler M, Osborn EA, Dewey CF Jr, McGrath JL, A mechanistic model of the actin cycle, *Biophys J* **86**:2720–2739, 2004.
62. Wriggers W, Schulten K, Stability and dynamics of G-actin: Back door water diffusion and behavior of a subdomain 3/4 loop, *Biophys J* **73**:624–639, 1997.

63. Wriggers W, Tang JX, Azuma T, Marks P, Janmey PA, Cofilin and gelsolin segment-1: Molecular dynamics simulation and biochemical analysis predict a similar actin binding mode, *J Mol Biol* **282**:921–932, 1998.
64. Kovar DR, Pollard TD, Insertional assembly of actin filament barbed ends in association with formins produces piconewton forces, *Proc Natl Acad Sci USA* **101**:14725–14730, 2004.
65. Balcer HI, Goodman AL, Rodal AA, Smith E, Kugler J, Heuser JE, Goode BL, Coordinated regulation of actin filament turnover by a high-molecular-weight Srv2/CAP complex, cofilin, profilin, and Aip1, *Curr Biol* **13**(24):2159–2169, 2003.
66. Volkmann N, Amann KJ, Stoilova-McPhie S, Egile C, Winter DC, Hazelwood L, Heuser JE, Li R, Pollard TD, Hanein D, Structure of Arp2/3 complex in its activated state and in actin filament branch junctions, *Science* **293**(5539):2456–2459, 2001.
67. Mercogliano CP, DeRosier DJ, Concatenated metallothionein as a clonable gold label for electron microscopy, *J Struct Biol* **160**(1):70–82, 2007.
68. Medalia O, Beck M, Ecke M, Weber I, Neujahr R, Baumeister W, Gerisch G, Organization of actin networks in intact filopodia, *Curr Biol* **17**(1):79–84, 2007.
69. Moseley JB, Goode BL, Differential activities and regulation of *Saccharomyces cerevisiae* formin proteins Bni1 and Bnr1 by Bud6, *J Biol Chem* **280**(30):28023–28033, 2005.
70. Miki H, Sasaki T, Takai Y, Takenawa T, Induction of filopodium formation by a WASP-related actin-depolymerizing protein N-WASP, *Nature* **391**:93–96, 1998.
71. Govind S, Kozma R, Monfries C, Lim L, Ahmed S, Cdc42Hs facilitates cytoskeletal reorganization and neurite outgrowth by localizing the 58-kD insulin receptor substrate to filamentous actin, *J Cell Biol* **152**(3):579–594, 2001.
72. Suetsugu S, Murayama K, Sakamoto A, Hanawa-Suetsugu K, Seto A, Oikawa T, Mishima C, Shirouzu M, Takenawa T, Yokoyama S, The RAC binding domain/IRSp53-MIM homology domain of IRSp53 induces RAC-dependent membrane deformation, *J Biol Chem* **281**(46):35347–35358, 2006.
73. Bompard G, Sharp SJ, Freiss G, Machesky LM, Involvement of Rac in actin cytoskeleton rearrangements induced by MIM-B, *J Cell Sci* **118**:5393–5403, 2005.
74. Eden S, Rohatgi R, Podtelejnikov AV, Mann M, Kirschner MW, Mechanism of regulation of WAVE1-induced actin nucleation by Rac1 and Nck, *Nature* **418**:790–793, 2002.

Natalia Sinitsina received her Diploma in Physiology from Lomonosov Moscow State University, Russia, in 2007. Since October 2007, she has been a Ph.D. student at Moscow State University. Her interests lie in the area of proteomics and the structure of actin-binding proteins.

Igor Orshansky received his Diploma in Biophysics from Lomonosov Moscow State University, Russia, in 2001. Since 2001, he has been a postgraduate student of the Bioengineering Department, Biological Faculty, Lomonosov Moscow State University. He is a member of the Russian Biochemical Society.

Olga Sokolova received her Diploma in Physiology from Lomonosov Moscow State University, Russia, in 1990, and her Ph.D. degree from the Russian Academy of Sciences in 1996. From 1997 to 1999, she was a postdoc at the University of Massachusetts, Boston, MA, USA; and from 1999 to 2000, at Brandeis University, Waltham, MA. In 2000, she was awarded the Jane Coffin Childs (and Agouron) postdoctoral fellowship. From 2002 to 2004, she was with the Howard Hughes Medical Institution, where she started to study the structure of actin-binding proteins. Since 2006, she has been at the Moscow State University, Faculty of Biology, as a group leader. Her group works on single-particle electron microscopy and the structure of large protein complexes.