The state of the filament

Adeleke H. Aguda¹, Leslie D. Burtnick² & Robert C. Robinson^{1*}

¹Uppsala University, Uppsala, Sweden, and ²The University of British Columbia, Vancouver, British Columbia, Canada

Movement is a defining characteristic of life. Macroscopic motion is driven by the dynamic interactions of myosin with actin filaments in muscle. Directed polymerization of actin behind the advancing membrane of a eukaryotic cell generates microscopic movement. Despite the fundamental importance of actin in these processes, the structure of the actin filament remains unknown. The Holmes model of the actin filament was published 15 years ago, and although it has been widely accepted, no high-resolution structural data have yet confirmed its veracity. Here, we review the implications of recently determined structures of F-actin-binding proteins for the structure of the actin filament and suggest a series of *in silico* tests for actin-filament models. We also review the significance of these structures for the arp2/3-mediated branched filament.

Keywords: actin filament; arp2/3; WH2; gelsolin; structure

EMBO reports (2005) 6, 220–226. doi:10.1038/sj.embor.7400363

The structure of the actin monomer

In this review, we examine the current understanding of the structures of the actin filament and the branched filament that is generated by actin-related protein 2/3 complex (arp2/3), and pose the question, “Do we know the structure of the actin filament?” Before building up these complex actin structures, it is worth asking the seemingly trivial question, “Do we know the structure of the actin monomer?” The answer to this question is surely “Of course we do.” After all, there are reports of high-resolution structures of actin bound to a myriad of sequestering agents: proteins such as DNase I (Kabsch *et al*, 1990), profilin (Chik *et al*, 1996; Schutt *et al*, 1993), gelsolin (Burtnick *et al*, 2004; McLaughlin *et al*, 1993; Robinson *et al*, 1999), vitamin-D-binding protein (DBP; Otterbein *et al*, 2002), and a hybrid between gelsolin domain 1 and thymosin-β4 (Irobi *et al*, 2004); small molecules such as macrolides (Klenchin *et al*, 2003); actin crosslinked to itself (Bubb *et al*, 2002) or rhodamine (Otterbein *et al*, 2001); and in combination with many binding partners such

as ciboulot/latrunculin (Hertzog *et al*, 2004) and the Bni1 formin homology domain 2 (FH2)/rhodamine (Otomo *et al*, 2005). These actin structures may be classified as ‘open’ (Fig 1B), as observed in a profilin:actin structure (Chik *et al*, 1996), or ‘closed’, as is seen for all others (Fig 1A), including a form of profilin:actin (Schutt *et al*, 1993). Actin consists of four subdomains that are arranged in pairs (Fig 1A–C) that sandwich the nucleotide and a metal ion. Minor differences can be seen between closed-form structures, which are most apparent in the conformation of a loop of subdomain 2 (D-loop) and slight variations in the angle between the two halves of actin. In the open form, subdomain 2 rotates away from subdomain 4 to open the nucleotide-binding cleft and leave a clear path for nucleotide exchange. Although the large number of known structures that have the closed conformation clearly suggest it to be the structure of the monomer, we should examine the set of structures in question and consider that most of the agents used to stabilize them also affect nucleotide exchange. With the exception of profilin, these agents inhibit nucleotide exchange probably by narrowing the nucleotide-binding cleft, and thus lock actin in the closed form. Therefore, the structure of the actin monomer in isolation is not known with absolute certainty. It may oscillate between the well-defined closed form and more open forms, such as the profilin:actin open structure, in order to exchange nucleotide (that is, move from the structure presented in Fig 1C through to that in Fig 1B and onto the one in Fig 1A in order to replace ADP with ATP). Despite uncertainties in the structure of the isolated actin monomer, we know the structures of profilin:actin (Chik *et al*, 1996; Schutt *et al*, 1993) and have a good idea of the Wiscott–Aldrich syndrome protein (WASP) homology 2 domain (WH2):actin structure. These are the biologically relevant forms of the actin monomer that are used by cells to feed filament elongation.

Do we know the structure of the actin filament?

Before addressing this issue, our definition of the term ‘structure’ should be established. Protein–protein interactions occur on a scale that requires the precise positioning of individual atoms at the interaction interface. Protein crystallography and nuclear magnetic resonance (NMR) can be used to determine structures at such a resolution. Methods that do not afford the appropriate resolution, or that lack certainty in their interpretation, produce three-dimensional representations that are designated as models rather than structures.

¹Department of Medical Biochemistry and Microbiology, Uppsala Biomedical Center, Uppsala University, Uppsala 751 23, Sweden

²Department of Chemistry and Centre for Blood Research, The University of British Columbia, Vancouver, British Columbia V6T 1Z1, Canada

*Corresponding author. Tel: +46 18 471 4933; Fax: +46 18 471 4975; E-mail: bob.robinson@imbim.uu.se

Submitted 23 November 2004; accepted 25 January 2005

Fig 1 | Actin and actin-related protein (arp) structures. (A) An example of the closed form of the actin monomer (Protein DataBase (PDB) code 2BTF). The nucleotide (ATP) is shown as a ball-and-stick representation and the metal ion as a green sphere. The subdomains are labelled 1–4 and are coloured yellow (1,2) and blue (3,4). (B) The open form of the actin monomer (PDB code 1HLU). (C) A second example of the closed structure of the actin monomer (PDB code 1J6Z). In this ADP-bound structure the distinctive D-loop is labelled. (D) A composite of Wiscott–Aldrich syndrome protein homology 2 (WH2) motif structures (red) in complex with actin (PDB codes 1T44 and 1SQK). (E) The N-terminal half of gelsolin (G1–G3, red) bound to actin (PDB code 1RGI). (F) The C-terminal half of gelsolin (G4–G6, red) bound to actin (PDB code 1H1V). (G) The Bni1 formin homology domain 2 (FH2) domain bound to actin (PDB code 1Y64). Only one-half of the FH2 dimer is depicted for clarity. (H) The arp2/3 complex (PDB code 1K8K). Arp2 (orange), arp3 (red), P40 (green), P34 (cyan), P21 (yellow) and P20 (blue) are visible in this orientation, while P16 (purple) is obscured from view. The missing portion of arp2 is modelled (Robinson *et al*, 2001). Asterisks represent the barbed ends of arp2 and arp3. An arrow indicates the direction in which arp2 is required to move to adopt a Holmes-like filament orientation with respect to arp3.

These include electron-diffraction models, fibre-diffraction models and models based on crystallographic contacts. So, the answer is “No”, we do not know the structure of the actin filament, but we do have several models. These models include the Holmes fibre-diffraction model (Holmes closed model; Holmes *et al*, 1990); the

Egelman electron microscopy reconstruction model (Belmont *et al*, 1999), which is a Holmes-like model that comprises open-cleft actin protomers (Holmes-like open model); and the unrelated ribbon-to-helix hypothesis that is based on actin:actin crystal contacts within profilin:actin crystals (Schutt *et al*, 1993).

Given that we do not know the structure of the filament, can the database of known actin monomer structures be used to distinguish the best filament models? Recently, several actin monomer structures have been determined involving proteins that are also able to interact with actin filaments. Some members of the WH2 family of proteins release their bound actin monomer during polymerization, and at least one, ciboulot, is able to cap the pointed end of the filament (Fig 1D; Hertzog *et al*, 2004; Irobi *et al*, 2004). Gelsolin is able to cap, sever and nucleate actin filaments and, under *in vitro* conditions, form complexes with actin monomers (Fig 1E,F; Burtnick *et al*, 2004; Robinson *et al*, 1999). Bni1 FH2 is able to nucleate and processively elongate actin filaments (Fig 1G; Otomo *et al*, 2005). Arp2/3 is a complex of seven proteins that includes two subunits, arp2 and arp3, each of which resembles actin in structure (Fig 1H; Robinson *et al*, 2001). The two arps are thought to adopt a filament-like orientation in order to act as the first two subunits in the arp2/3 branched daughter filament. If we assume that the interactions of these filament-binding proteins with actin, or with the arps, will be similar to a first approximation, whether they are bound to a monomer or to a filament, these structures have relevance in the evaluation of actin filament models. However, it should be noted that at least two conformational changes are expected in the actin monomer structure on incorporation into a filament. The first, which occurs immediately on addition of actin monomers to filaments, is evinced by the release of profilin and the increased rate of ATP hydrolysis (Blanchoin & Pollard, 2002). The second, which follows hydrolysis of ATP and the release of phosphate, is seen by the preference of many filament-binding proteins for filaments that bear a particular nucleotide. Furthermore, many actin-binding proteins, including gelsolin, are known to cause conformational changes in the filament (Orlova *et al*, 1995). Hence, in the analyses below, we allow moderate conformational changes in the actin-protomer structure and also permit small changes in the protomer orientation.

Interactions across the filament

Gelsolin, ciboulot and arp2/3 are able to cap actin filaments, and gelsolin, Bni1 FH2 and arp2/3 can nucleate filaments (Burtnick *et al*, 2001; Dayel & Mullins, 2004; Hertzog *et al*, 2002; Otomo *et al*, 2005). Therefore, the actin-monomer-bound forms of ciboulot, gelsolin and FH2 must allow for the formation of actin:actin contacts across the filament, while obscuring the appropriate filament end. Similarly, within the arp2/3 complex, the interactions of the accessory proteins with arp2 and arp3 should allow arp2 and arp3 to adopt an orientation appropriate to an actin filament. Superposition of the actin structures from the G1–G3, G4–G6, FH2 and the WH2 domain actin complexes, and overlaying arp2 and arp3 onto actin with their accessory proteins P40, P16 and P20, and P21 and P34, respectively, provides a picture of the external face of an actin filament (Fig 2A–D). These filament-binding proteins extensively cover one face and the two sides of an actin protomer, while leaving its opposite face free. The unobscured face represents the surface that is available for actin–actin interactions across the filament and provides the first test of validity for the filament models. In the Holmes-like models, the actin–actin interaction footprint is buried in the cross-filament interactions (Fig 2D), which satisfies this test. By default, models that are grossly different to the Holmes model are contrary to this analysis.

Biology of the interacting proteins

Relations between the structures and biology of the F-actin-binding proteins that are discussed above provide further tests for models of the actin filament. The WH2 motif superfamily includes the β -thymosin subfamily, members of which sequester actin monomers and do not directly release them for filament elongation. The WH2 domain structures show that the two termini of thymosin- β 4 are able to hinder its inclusion at either the barbed or the pointed end of a Holmes-like filament (Hertzog *et al*, 2004; Irobi *et al*, 2004). Two modifications exist that turn the sequestering β -thymosin into the more general WH2 barbed-end elongating module. First, the carboxyl terminus is shortened or its actin binding is impaired to allow inclusion of WH2:actin at the barbed end of the filament (Hertzog *et al*, 2004). Second, proteins that contain many WH2 motifs are consistent with decorating neighbouring actin subunits on one face of the Holmes-like models, and during elongation may deliver actin monomers to the barbed end (Irobi *et al*, 2004).

When the structures of the two halves of gelsolin bound to actin are superimposed onto the Holmes closed model of the filament, steric clashes occur that involve gelsolin domains G1 and G4 with the neighbouring longitudinally associated actin protomers, in line with the capping function of these domains (Burtnick *et al*, 2004). Furthermore, G2, the F-actin-binding domain, is orientated with respect to a third actin to allow their predicted binding surfaces to come together (Choe *et al*, 2002; Puius *et al*, 2000). Hence, the Holmes-like models show no disparity with the gelsolin structures.

The Bni1 FH2:actin structure provides a powerful indication as to the mechanisms of nucleation, capping and elongation by the FH2 domain from the formin family of proteins (Otomo *et al*, 2005). Each FH2 chain contacts three actin monomers to create a filament nucleus. The conformation of the FH2 domain in the crystal prevents addition of a Holmes-like barbed-end actin through steric clashes. However, domain swapping by FH2 suggests how this steric hindrance may be overcome to allow processive elongation. Indeed, the crystals contain a pseudo-Holmes-like filament that is decorated by an FH2 polymer formed through domain swapping.

Finally, the structure of the arp2/3 complex shows that the relative positions of arp2 and arp3 would allow them to be moved into a Holmes-like orientation without disrupting the integrity of the remaining five subunits (Figs 1H, 3A,B; Robinson *et al*, 2001). Closer inspection reveals that the D-loop of arp2 would clash with P21 if an ADP-actin-like conformation were adopted by arp2 (Fig 1C). This clash does not occur if arp2 adopts an ATP-actin-like conformation. The structure of the ATP-bound closed form of arp3 (within arp2/3) was recently determined, and it is reasonable to assume that arp2 will also adopt an ATP-bound closed form during activation (Nolen *et al*, 2004). Hence, the arp2/3 structure provides a stern test for the Holmes-like models.

Given that the biological activity of the filament-binding proteins can be superficially accounted for by a filament model that is composed of the actin monomer structure, we expect that only subtle changes in the actin protomer structure will occur on polymerization.

Holmes-like models: open versus closed protomers

The Holmes closed model and the Holmes-like open model conflict in one regard: namely, the width of the nucleotide-binding cleft (Belmont *et al*, 1999; Holmes *et al*, 1990). The high-resolution structures under consideration here provide data relevant to this controversy. First, thymosin- β 4 interacts with an actin monomer by

Fig 2 | The actin–actin interaction footprint. (A) Superposition of arp2 (with its accessory proteins P40, P20 and P16), arp3 (with P34 and P21) and the actin complexes from Fig 1D–G onto a single actin monomer. The binding proteins are shown in red and actin is presented in the same orientation as in Fig 1A. This face of actin is almost completely obscured by the binding proteins. (B) A 180° rotation of (A) around the y-axis. This face of actin is free of binding proteins and available for actin–actin interactions. (C) Rotation of (A) to a position looking down onto the nucleotide-binding cleft (the pointed end). (D) The same as (C), but with a second actin from the Holmes model of the actin filament (Holmes *et al*, 1990). The Holmes orientated actin is located at the actin–actin interaction footprint.

inserting its C-terminal helix between actin subdomains 2 and 4. This binding surface would be abolished in the open form of actin. Hence, for related WH2 proteins to cap the filament through this interaction, the terminal pointed-end actin protomer is required to be in a closed conformation. Second, gelsolin caps the barbed end of a filament and secures the gelsolin-bound actins in a closed conformation by inserting a helix between actin subdomains 1 and 3, thereby preventing actin from adopting an open form. The F-actin-binding domain (G2) also requires a closed conformation in the

terminal actin to allow it access to its binding site on the longitudinally adjacent actin (Burnick *et al*, 2004). Thus, both ends of capped filaments require actin protomers that adopt a closed conformation. Furthermore, this argument may be extended to the centre of the filament. In order for gelsolin to bind to the side of a filament and initiate severing, G2 must bind to two actin protomers, one of which must be in the closed conformation to provide the correct binding surface for G2. Therefore, these structures support Holmes-like models that include at least a proportion of closed actin protomers.

Fig 3 | Models of arp2/3 conformational changes and branching. (A) Model of arp2/3 with bound C-motif (grey, labelled C). The C-motif was modelled by positioning the N-terminal half of a WH2 motif onto arp2 in the same manner in which it interacts with actin (Irobi *et al*, 2004). (B) Rotation model. Arp2/P40/P16/P20 have been rotated relative to arp3/P34/P21 to allow arp2 to adopt a Holmes filament orientation relative to arp3. (C) Arp2 migration model. Arp2 has been moved into a Holmes filament orientation relative to arp3. (D) Model of an arp2/3-mediated branch. The arp2/3 model from (C) has been rotated and two actin:WH2 motif structures docked onto arp2 in a Holmes filament orientation. The two WH2 motifs, as found in N-WASP are labelled V1 and V2, and the C region is indicated. The arrow suggests the flat surface to which the mother filament may bind, creating the 70° branch. Asterisks denote the barbed end of the filaments and 'x' marks the positions of joining actin monomers.

The arp2/3 branched filament

The recent structures of the WH2-motif:actin complexes have provided new insights into the activating proteins for the arp2/3-mediated branch. The arp2/3 activators—the WASP family of proteins—contain a minimal activating motif, VCA, that interacts both with actin (V, a WH2 motif) and arp2/3 (CA; Panchal *et al*, 2003). The structures of ciboulot and thymosin- β 4 define the portion of VCA that contacts an actin monomer (Hertzog *et al*, 2004; Irobi *et al*, 2004). In the double-WH2-motif-containing protein N-WASP, the proximity of the two V motifs limits them to assembly with two longitudinally related actin protomers during nucleation. The structures of ciboulot and thymosin- β 4 also led both sets of authors to predict that the amphipathic helix in the C region of the WASP family of proteins will interact with arp2 in a manner similar to that between the amino-terminal helix of the WH2 motifs and actin. The implication, again due to proximity considerations, is that the VCA motif will dock the V-bound actin onto arp2. This idea is corroborated by the activity of a hybrid arp2/3 in which arp2 was fused to a V motif; the hybrid protein supported branch formation in the presence of CA (Goley *et al*, 2004).

The arp2/3 binding sites for CA are widespread and are found on arp2, arp3, P40 and P21 (Zalevsky *et al*, 2001). Recently, recombinant P40 was shown to contain a high-affinity binding site for VCA that is roughly equivalent to that of the entire arp2/3 complex. This contact includes P40 interacting with the A region (Pan *et al*, 2004), which was previously shown to bind to arp3 and P21 (Machesky & Insall, 1998; Weaver *et al*, 2002). Given the proposed docking of the V-bound actin onto arp2 and the high-affinity interaction of CA with P40, it is difficult to reconcile the distances between arp2/P40 and arp3/P21 with the chain length of CA (the length of the amphipathic helix + 24 residues in the case of WASP-family verprolin homologous protein 1 (WAVE1)). Conformational change in arp2/3 provides at least a partial explanation (Goley *et al*, 2004). However, a dynamic role for CA may also be considered, in which CA may destabilize the inactive conformation through binding to one site on arp2/3 (for example, P40) and initiate a conformational change. The active conformation of arp2/3 may then offer a competing CA-binding site (perhaps arp3/P21) that would stabilize active arp2/3 once occupied.

Two structural rearrangements of arp2/3 have been suggested that will bring arp2 and arp3 into a Holmes-like alignment in order to provide the nucleus for the daughter filament. The rotation model predicts that the two halves of arp2/3 (arp3/P21 versus arp2/P16/P40; Robinson *et al*, 2001) will rotate relative to each other on the backbone of P20/P34 (Fig 3A,B). Alternatively, the arp2-migration model suggests that interaction between P40 and arp2 is released, which would allow arp2 to be delivered to arp3 by the N-terminal extension of P16 (Fig 3A,C; Irobi *et al*, 2004). Either mechanism allows for the construction of models of the arp2/3 branch solely through structural superposition of known structures and the Holmes model (Fig 3D). The flat face of the arp2/3 complex makes a natural angle of about 70° with the daughter branch, which suggests the position of the mother filament.

In conclusion, recent structures of F-actin-binding proteins have allowed us to reassess filament models. These structures are consistent with a Holmes-like filament that comprises, at least in part, closed-cleft protomers. The structures of arp2/3 and the WH2 motif in complex with actin have led to possibilities for the construction of the arp2/3 branched filament. However, these predictions are only models and more structural data are required to determine their worth.

ACKNOWLEDGEMENTS

The EMBO Young Investigator scheme and the Swedish Medical Research Council support R.C.R. L.B. thanks the Heart and Stroke Foundation of British Columbia and Yukon for support.

REFERENCES

- Belmont LD, Orlova A, Drubin DG, Egelman EH (1999) A change in actin conformation associated with filament instability after Pi release. *Proc Natl Acad Sci USA* **96**: 29–34
- Blanchoin L, Pollard TD (2002) Hydrolysis of ATP by polymerized actin depends on the bound divalent cation but not profilin. *Biochemistry* **41**: 597–602
- Bubb MR, Govindasamy L, Yarmola EG, Vorobiev SM, Almo SC, Somasundaram T, Chapman MS, Agbandje-McKenna M, McKenna R (2002) Polylysine induces an antiparallel actin dimer that nucleates filament assembly: crystal structure at 3.5-Å resolution. *J Biol Chem* **277**: 20999–21006
- Burnick LD, Robinson RC, Choe S (2001) Structure and function of gelsolin. *Results Probl Cell Differ* **32**: 201–211
- Burnick LD, Urosov D, Irobi E, Narayan K, Robinson RC (2004) Structure of the N-terminal half of gelsolin bound to actin: roles in severing, apoptosis and FAF. *EMBO J* **23**: 2713–2722
- Chik JK, Lindberg U, Schutt CE (1996) The structure of an open state of β -actin at 2.65 Å resolution. *J Mol Biol* **263**: 607–623
- Choe H, Burnick LD, Mejillano M, Yin HL, Robinson RC, Choe S (2002) The calcium activation of gelsolin: insights from the 3 Å structure of the G4–G6/actin complex. *J Mol Biol* **324**: 691–702
- Dayel MJ, Mullins RD (2004) Activation of Arp2/3 complex: addition of the first subunit of the new filament by a WASP protein triggers rapid ATP hydrolysis on Arp2. *PLoS Biol* **2**: E91
- Goley ED, Rodenbusch SE, Martin AC, Welch MD (2004) Critical conformational changes in the arp2/3 complex are induced by nucleotide and nucleation promoting factor. *Mol Cell* **16**: 269–279
- Hertzog M, Yarmola EG, Didry D, Bubb MR, Carlier MF (2002) Control of actin dynamics by proteins made of β -thymosin repeats: the actobindin family. *J Biol Chem* **277**: 14786–14792
- Hertzog M *et al* (2004) The β -thymosin/WH2 domain: structural basis for the switch from inhibition to promotion of actin assembly. *Cell* **117**: 611–623
- Holmes KC, Popp D, Gebhard W, Kabsch W (1990) Atomic model of the actin filament. *Nature* **347**: 44–49
- Irobi E, Aguda AH, Larsson M, Guerin C, Yin HL, Burnick LD, Blanchoin L, Robinson RC (2004) Structural basis of actin sequestration by thymosin- β 4: implications for WH2 proteins. *EMBO J* **23**: 3599–3608
- Kabsch W, Mannherz HG, Suck D, Pai EF, Holmes KC (1990) Atomic structure of the actin:DNase I complex. *Nature* **347**: 37–44
- Klenchin VA, Allingham JS, King R, Tanaka J, Marriott G, Rayment I (2003) Trisoxazole macrolide toxins mimic the binding of actin-capping proteins to actin. *Nat Struct Biol* **10**: 1058–1063
- Machesky LM, Insall RH (1998) Scar1 and the related Wiskott–Aldrich syndrome protein, WASP, regulate the actin cytoskeleton through the Arp2/3 complex. *Curr Biol* **8**: 1347–1356
- McLaughlin PJ, Gooch JT, Mannherz HG, Weeds AG (1993) Structure of gelsolin segment 1–actin complex and the mechanism of filament severing. *Nature* **364**: 685–692
- Nolen BJ, Littlefield RS, Pollard TD (2004) Crystal structures of actin-related protein 2/3 complex with bound ATP or ADP. *Proc Natl Acad Sci USA* **101**: 15627–15632
- Orlova A, Prochiewicz E, Egelman EH (1995) Structural dynamics of F-actin. II. Cooperativity in structural transitions. *J Mol Biol* **245**: 598–607
- Otomo T, Tomchick DR, Otomo C, Panchal SC, Machius M, Rosen MK (2005) Structural basis of actin filament nucleation and processive capping by a formin homology 2 domain. *Nature* **433**: 488–494
- Otterbein LR, Graceffa P, Dominguez R (2001) The crystal structure of uncomplexed actin in the ADP state. *Science* **293**: 708–711
- Otterbein LR, Cosio C, Graceffa P, Dominguez R (2002) Crystal structures of the vitamin D-binding protein and its complex with actin: structural basis of the actin-scavenger system. *Proc Natl Acad Sci USA* **99**: 8003–8008

- Pan F, Egile C, Lipkin T, Li R (2004) ARPC1/Arc40 mediates the interaction of the actin-related protein 2 and 3 complex with Wiscott–Aldrich syndrome protein family activators. *J Biol Chem* **279**: 54629–54636
- Panchal SC, Kaiser DA, Torres E, Pollard TD, Rosen MK (2003) A conserved amphipathic helix in WASP/Scar proteins is essential for activation of Arp2/3 complex. *Nat Struct Biol* **10**: 591–598
- Puius YA, Fedorov EV, Eichinger L, Schleicher M, Almo SC (2000) Mapping the functional surface of domain 2 in the gelsolin superfamily. *Biochemistry* **39**: 5322–5331
- Robinson RC, Mejillano M, Le VP, Burtnick LD, Yin HL, Choe S (1999) Domain movement in gelsolin: a calcium-activated switch. *Science* **286**: 1939–1942
- Robinson RC, Turbedsky K, Kaiser DA, Marchand JB, Higgs HN, Choe S, Pollard TD (2001) Crystal structure of Arp2/3 complex. *Science* **294**: 1679–1684
- Schutt CE, Myslik JC, Rozycki MD, Goonesekere NC, Lindberg U (1993) The structure of crystalline profilin- β -actin. *Nature* **365**: 810–816
- Weaver AM, Heuser JE, Karginov AV, Lee WL, Parsons JT, Cooper JA (2002) Interaction of cortactin and N-WASP with Arp2/3 complex. *Curr Biol* **12**: 1270–1278
- Zalevsky J, Lempert L, Kranitz H, Mullins RD (2001) Different WASP family proteins stimulate different Arp2/3 complex-dependent actin-nucleating activities. *Curr Biol* **11**: 1903–1913

Adeleke H. Aguda

Leslie D. Burtnick

Robert C. Robinson is the recipient of an EMBO Young Investigator Award