Appendix 3

Turning on the Arp2/3 Complex at Atomic Resolution

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Turning on the Arp2/3 Complex at Atomic Resolution

Review

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The recently published 2 Å X-ray crystal structure of bovine Arp2/3 complex gives us atomic scale insight into Arp2/3-mediated actin nucleation, while cryo-EM work and functional studies begin to fill in exciting mechanistic details.

Diverse cellular processes including shape change and crawling locomotion require the rapid and precise generation of actin filament arrays. The polymerization and organization of actin is therefore highly regulated both spatially and temporally by dozens of actin-associated proteins. In this delicately balanced system, the rate-limiting step for actin polymerization is the creation of new barbed ends to which actin monomers can add. New barbed ends can be created from existing filaments by uncapping or severing, or from actin monomers by de novo nucleation. While uncapping and severing both play important roles, there has been much recent research into the mechanism of the de novo nucleation of actin filaments in response to cellular signals (reviewed in [1]). In particular, many studies have focused on the Arp2/3 complex, the key to the molecular mechanism of de novo nucleation in cells.

Though of modest molecular weight (~220 kDa), the Arp2/3 complex is a structurally unique protein consisting of seven different polypeptides (Table 1) with nanomolar affinity for one another (reviewed in [2]). These seven subunits range in size from about 16 to a little less than 50 kDa and have been conserved in sequence and function from yeast to man. The two largest subunits, actin-related proteins Arp2 and Arp3, give the complex its name [3, 4]. Based on structural modeling, Arp2 and Arp3 were predicted to fold and bind ATP like actin does [5]. Moreover, the two Arps were proposed to form an actin-like dimer to which a single actin monomer or dimer need bind to create an actin nucleus competent for rapid elongation [5]. In fact the Arp2/3 complex on its own has little actin-nucleating activity, but it can be activated to nucleate actin polymerization (Figure 1) by a class of proteins called nucleation promoting factors (NPFs). Multiple families of NPFs have now been identified including the *Listeria monocytogenes* ActA protein, WASP/N-WASP/Scar proteins, cortactin, Abp1p, yeast type I myosins, and Pan1p (reviewed in [6, 7]). The best-characterized NPFs, the WASP family proteins, are multidomain proteins that bind Arp2/3 complex and monomeric actin as well as upstream signaling molecules [6]. By binding to both Arp2/3 complex and signaling factors, NPFs can control where and when new actin filaments are created in the cell.

Interestingly, the activity of an Arp2/3 complex•NPF•actin assembly is further stimulated by binding to the side of an existing actin filament (Figure 1) [8]. By binding to the side of this "mother" filament, nucleating the formation of a new "daughter" filament, and remaining bound to that new filament, activated Arp2/3 complex organizes actin into "Y-branches" that maintain a characteristic angle of 70° [9]. The formation of Arp2/3 complex mediated Y-branches has recently been observed in real time in vitro [10]. Moreover, Arp2/3 complex is localized to the leading edge of moving cells [11, 12], where it appears to mediate the formation of Y-branches [13].

So how do all seven subunits of the Arp2/3 complex work together to perform these various functions? Since the nucleation activity of the Arp2/3 complex can be turned on and off without apparent dissociation or association of subunits, a structural change must take place within the complex during the conversion between the active and inactive states. Arp2/3 complex practitioners now have their first structural insight into the mechanism of activation. Both a cryo-EM reconstruction of Arp2/3 in Y-branches [14] as well as a 2Å X-ray crystal structure of bovine Arp2/3 complex [15] have now been published. While beautifully answering some basic questions, both of these studies leave one thirsting for more functional information. Fortunately, functional details about the activation of Arp2/3 complex have also been emerging.

An Atomic Resolution Picture of Arp2/3 Complex

Many techniques have been used to examine the interactions between subunits in the Arp2/3 complex, including chemical crosslinking [16], two-hybrid assays [17, 18], genetic studies [19, 20], and most recently reconstitution of the complex from individual subunits expressed in insect cells [21]. A model of the Arp2/3 complex that synthesizes all the available genetic and biochemical data on subunit-subunit interactions is presented in Figure 2A [21]. We can now compare this model to the 2Å resolution crystal structure of bovine Arp2/3 complex determined by Robinson et al. (Figures 2B and 3A, top). The crystallographic model, with R_{cryst} and R_{free} refined to 21.6% and 24.9%, respectively, incorporates 86% of the 1980 residues in the complex. When the crystal structure is compared to the biochemical model, the correspondence is excellent. A notable exception is the interaction of ARPC3 with Arp2 and ARPC4 (based on genetic and two-hybrid data) shown in the model but not evident in the crystal structure. These data should not be dismissed, however, because it is possible that at least one of these contacts exists when the complex is in another conformation.

Two-hybrid interactions initially suggested that ARPC2 and ARPC4 would interact [17, 18], and data from genetic analysis [20] and Arp2/3 complex reconstitution

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Table 1. Arp2/3 Complex Nomenclature

Standard Name	ACTR2	ACTR3	ARPC1	ARPC2	ARPC3	ARPC4	ARPC5
Mammalian protein	Arp2	Arp3	p41-Arc	p34-Arc	p21-Arc	p20-Arc	p16-Arc
Amoeba protein	Arp2	Arp3	p40	p35	p18	p19	p14
Budding yeast protein	Arp2	Arp3	Arc40	Arc35	Arc18	Arc19	Arc15

[21] predicted that these two subunits would form a heterodimer at the structural "core" of the complex. These predictions are borne out in the crystal structure, where ARPC2 and ARPC4 are the most buried subunits in the complex, with over 2000 Å^2 of surface area packed between them and a further 5000 Å^2 packed against Arp2, Arp3, ARPC1, and ARPC5. Interestingly, ARPC2 has two modules and ARPC4 has one module of the same previously unrecognized α/β-fold, though there is very little sequence identity among these repeated structural elements. ARPC3 and ARPC5 are more peripherally located and also have previously unrecognized, primarily α-helical folds. ARPC1 contains WD repeats and adopts a seven-bladed β propeller fold, as was predicted from its sequence.

The Structure of Arp2 and Arp3—A Mechanism for Nucleation

Because of their similarity to actin, speculation about the structure of Arp2 and Arp3 has been extensive. Do they form a template onto which actin monomers can add? While the answer seems to be yes, we still do not know the exact structure of the template. The crystal structure determined by Robinson et al. almost certainly represents an inactive complex. However, as long as a few reasonable conformational changes occur, there is no apparent impediment to Arp2 and Arp3 coming together in an actin-like dimer that templates filament formation. The crystal structure of actin fits into the electron density for Arp3 quite well except that the nucleotide binding cleft of Arp3 is open 12° further than actin and no nucleotide is bound. Both Arp2 and Arp3 also have insertions in their amino acid sequences compared to actin. Structural modeling predicted that all insertions would fall on the surface of the proteins and would not disturb the core fold [5]. In the case of Arp3, these predictions were right on target. Arp3's four large insertions do indeed fall on the surface, generally extending loops and/or adjacent elements of secondary structure. One insertion creates a binding site for ARPC3 while the others are primarily exposed to solvent. The exact structure of Arp2 is less clear because, while half of the molecule is bound by four other subunits and shows strong density, there is no strong density for the other half. However, a polyalanine model based on actin was oriented in weak density that appeared after several rounds of refinement and the result indicated that the nucleotide binding cleft of Arp2 was also open with no nucleotide bound.

ATP binding and/or hydrolysis are required for the full activation of Arp2/3 complex [22, 23]. Therefore Arp2 and Arp3 must bind ATP and close their nucleotide binding clefts as part of the activation step. In addition, surface features on Arp3 have to be rearranged for a proper template to form [15]. However, even with these structural transformations, a larger conformational change is needed before Arp2 and Arp3 would assume an actin dimer-like orientation. As Arp2 and Arp3 are "face-to-face" in the inactive structure, activation could entail a modest rigid body rotation of Arp2, ARPC4, ARPC1, and ARPC5 relative to Arp3, ARPC2, and ARPC3, bringing Arp2 and Arp3 into the same conformation as a short-pitch dimer in an actin filament with Arp2 at the barbed end (Figure 3A) [15]. Robinson et al. assert that only this particular Arp2/Arp3 dimer is structurally reasonable and that all complex activators likely stabilize this conformation. Interestingly, though the Arp2/3 complex nucleates actin filaments that elongate only in the barbed-end direction, neither the structure of Arp2 and Arp3, nor their arrangement in the complex, seems to preclude pointed end growth. An explanation for this selectivity must therefore await the structure of the activated complex.

Mechanism of Activation and Side Binding

While the structure of the Arp2/3 complex suggests a very reasonable and beautiful mechanism of activation,

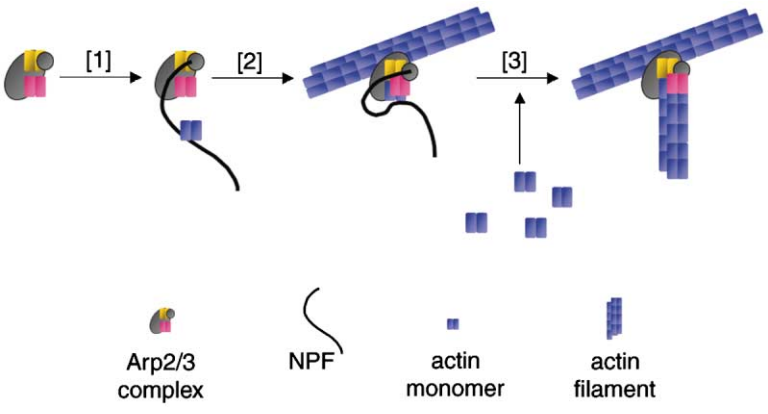

Figure 1. Actin Filament Nucleation and Organization by Arp2/3 Complex

Step 1: An actin monomer-bound NPF molecule (WASP family or ActA) associates with inactive Arp2/3 complex.

Step 2: The NPF•actin•Arp2/3 complex assembly binds to the side of an actin filament and becomes fully activated.

Step 3: Actin monomers rapidly add onto the barbed end template created by the Arp2/3 complex, creating a new Y-branch.

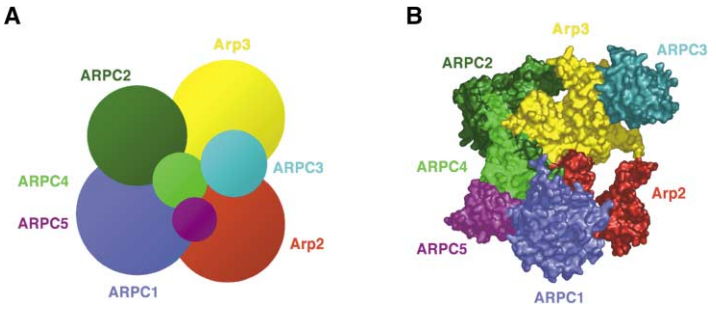

Figure 2. Models of the Arp2/3 Complex

(A) Model based on biochemical, crosslinking, two-hybrid, and genetic data. Modified from [21].

(B) Space-filling model of the 2 Å crystal structure of bovine Arp2/3 complex [15]. Coordinates kindly provided by Bob Robinson, Kirsi Turbedsky, and Tom Pollard.

how NPFs and actin filaments bind to the complex and effect the transformation is unresolved. It is tempting to speculate that the acidic C-terminal domain of WASP family NPFs binds to two large basic patches on ARPC1 and ARPC3, bridging the complex and bringing Arp2 and Arp3 into proximity [15]. In support of this model, the NPFs ActA and Scar1 can be chemically crosslinked to Arp2, Arp3, and ARPC1 [24]. However, Gournier et al. show that complexes lacking ARPC3 or ARPC5 and ARPC1 can still be stimulated by the NPFs ActA and WASP, albeit less robustly than the intact complex, suggest that the mechanism of activation is complex [21]. In addition to this complication, it has been shown that various NPFs, including different members of the WASP family [25, 26], do not all activate Arp2/3 complex equally well. Determining the structure of Arp2/3 complex bound to each class of NPF should help us understand these differences in detail.

In addition to the mechanism of activation, the structure of the Arp2/3 complex bound to mother and daughter actin filaments is beginning to be addressed by cryo-electron microscopy (Figure 3B) [14]. Because the helicity of the mother filament is undisturbed by complex binding and because the mass of protein between the two helical filaments in the branch is too small to contain all seven subunits, Volkman et al. conclude that the first two monomers in the daughter filament must be Arp2 and Arp3. The complex appears to be attached to the side of the mother filament via three bridges of density contacting three actin molecules. However, because none of the non-Arp subunits of the complex resemble any known actin filament binding protein, it is hard to

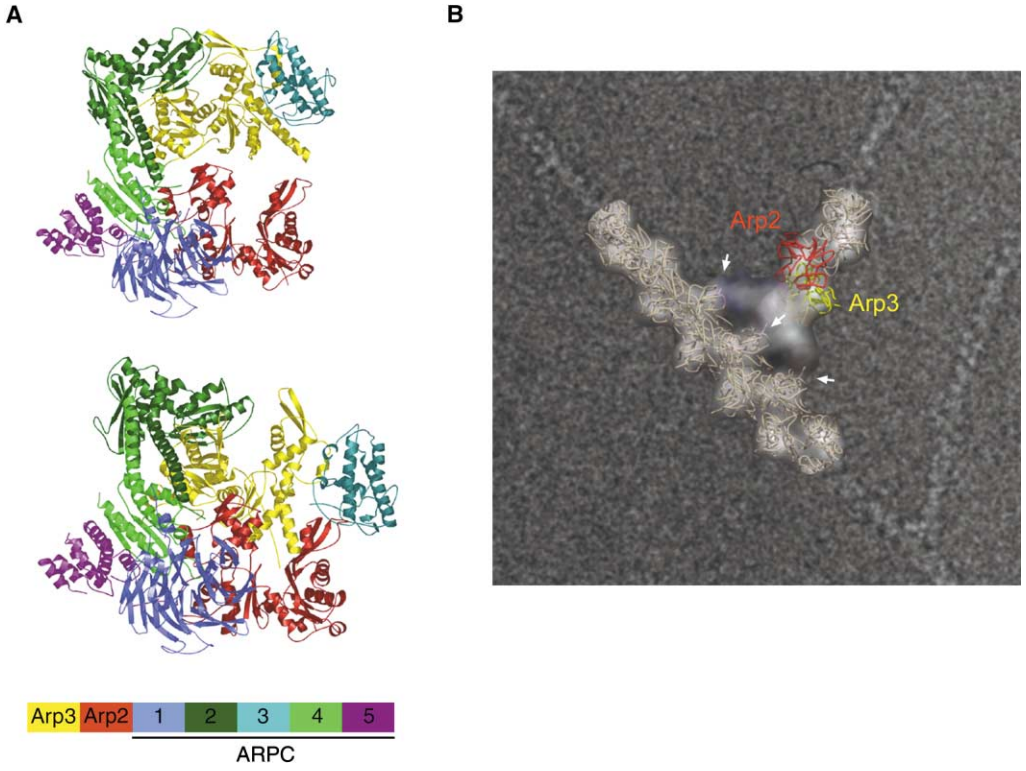

Figure 3. Arp2/3 Complex in the Activated State

(A) A comparison of the inactive Arp2/3 complex (top) and a model for the activated conformation (bottom). Modified from [15].

(B) Model of Arp2/3 complex in the Y-branch. A molecular model of F-actin is fitted into a 2D reconstruction of the Y-branch determined by electron microscopy [14]. Arrows indicate the three bridges of density observed to connect the Arp2/3 complex to the mother filament. Image kindly provided by Niels Volkmann and Dorit Hanein.

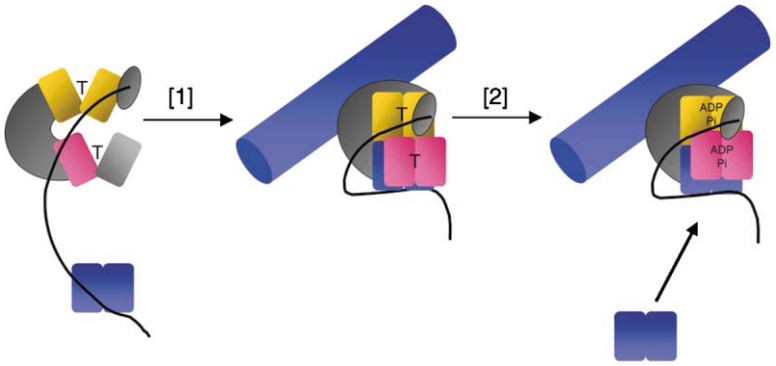

Figure 4. Schematic Diagram of Arp2/3 Complex Activation

Step 1: Inactive Arp2/3 binds to ATP and to a complex of NPF•actin, closing the nucleotide binding clefts of Arp2 and Arp3. The actin monomer bound to the NPF associates with Arp2 and Arp3 and the entire assembly binds to the side of a mother filament.

Step 2: ATP is hydrolyzed by Arp2 and/or Arp3 and elongation ensues.

predict which subunit constitutes the mother filament binding interface. Taking into consideration crosslinking, biochemical, and genetic data, Volkmann, et al. propose that ARPC1, ARPC2, and ARPC5 form these bridges. A critical role for ARPC2 is further supported by the fact that isolated ARPC2/ARPC4 heterodimers can bind to actin filaments [21]. In support of a role for ARPC1, Robinson et al. note that a helix formed by residues between blades 6 and 7 of the ARPC1 β propeller contacts Arp3 of a neighboring complex in the crystal lattice. In the end, however, understanding how the Arp2/3 complex interacts with and is activated by actin filaments will come only after further structural studies of filament-bound Arp2/3 complex.

The Role of ATP Binding and Hydrolysis

It has recently been suggested that, even after an Arp2/3 complex•NPF•actin assembly binds the side of a filament, another step having first-order kinetics must occur before a competent nucleus is formed [26, 27]. Two new papers predict that this step involves ATP hydrolysis (Figure 4) [22, 23]. Although both groups find that Arp2 and Arp3 bind ATP and that binding of the NPF N-WASP increases the affinity of at least one of the two Arps for ATP, they differ in some details. If it is shown that NPF binding facilitates closure of the Arp2 and/or Arp3 nucleotide binding clefts (Figure 4), this may account for the increase in affinity. Even more exciting is the fact that both groups suggest that ATP hydrolysis by at least one of the Arps is required for Arp2/3-mediated nucleation and branching. The basal rate of ATP hydrolysis by Arp2/3 complex is extremely low and does not appear to be stimulated by NPFs [22, 23]. This suggests a model (Figure 4) in which ATP hydrolysis and nucleation occur after the Arp2/3 complex•NPF•actin assembly binds to the side of a mother filament.

Conclusion

The crystal structure of the Arp2/3 complex has given us atomic scale insight into Arp2/3-mediated actin nucleation while cryo-EM work and functional studies are beginning to fill in the mechanistic and physical details. It will be exciting to follow the results of further structural and functional studies aimed at elucidating the differences between Arp2/3 complexes bound to different NPFs, in various nucleotide states and in the activated state. A detailed atomic level description of the most exciting structure, the activated Arp2/3 complex assembled with an NPF and actin, poised to give birth to a daughter filament, may be some time coming, however. It is often difficult to freeze an enzyme in its transition state, but to do so in the presence of a self-polymerizing molecule like actin is truly a technical challenge. Perhaps the powerful combination of cryo-electron microscopy and X-ray crystallography will soon give us a close up look at this fascinating complex at work within the actin polymerization machinery.

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