Peering deeply inside the branch

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The actin-related protein 2/3 (Arp2/3) story has captivated the cytoskeleton community for over a decade. Not only does this complex nucleate new actin filaments, but it also anchors them into a dendritic meshwork that is used in many cellular contexts such as lamellipodial protrusion, endosome rocketing, and the movement of pathogens. One key piece of this puzzle that has been missing is a detailed structure of the Arp2/3-actin branch. Using electron tomography and computational docking, Rouiller et al. (Rouiller, I., X.-P. Xu, K.J. Amann, C. Egile, S. Nickell, D. Nicastro, R. Li, T.D. Pollard, N. Volkmann, and D. Hanein. 2008. J. Cell Biol. 180:887–895) present an elegant and intriguing structure of the Arp2/3 complex-mediated actin branch.

The dynamic reorganization of actin filaments plays a critical role in many cellular processes such as cell migration, endocytosis, formation of cell–cell junctions, and cell–matrix adhesion. Although many steps in actin filament reorganization are precisely regulated, one step that must be carefully controlled is the nucleation of new filaments. Several proteins and protein complexes have been identified that nucleate new actin filaments such as various formins, Spire, Cordon Bleu, and the Arp2/3 complex (Pollard, 2007; Winckler and Schafer, 2007). All of these mechanisms achieve actin filament nucleation, but the Arp2/3 complex is unique in that it anchors these new daughter filaments to the side of a preexisting mother filament at a characteristic ~70° angle.

This generation of actin branches by the Arp2/3 complex forms the basis for the dendritic nucleation model of actin assembly (Mullins et al., 1998). This type of actin assembly has been observed at the leading edge of many motile cell types as well as at sites of endocytosis and in the rocketing comet tails of pathogenic organisms such as Listeria monocytogenes (Goley and Welch, 2006). The universality of Arp2/3-based actin assembly has recently been called into question by a study that finds that actin-based processes continue to occur in the absence of Arp2/3 function (Gupton et al., 2005). This likely reflects the complexity and partial redundancy of actin assembly mechanisms in robust biological systems. However, the importance of Arp2/3 function is underscored by the recent discovery that deletion of genes encoding two of the complex subunits leads to preimplantation lethality in mice (Yae et al., 2006; Vauti et al., 2007).

The structure of the soluble, inactive Arp2/3 complex was solved in 2001 (Robinson et al., 2001). Although these studies were very informative, they did not give a precise picture of how the complex was oriented within the branch and did not provide information on how the mother filament might be modified at the branch.

Rouiller et al. (see p. 887 of this issue) provide a major step forward in this field by reporting the 3D structure of an Arp2/3-actin branch. The authors used both negative staining and cryo-EM (frozen-hydrated samples) along with tomographic reconstruction to visualize the structure of actin filament branches generated by the Arp2/3 complex. Impressively, they used Arp2/3 complex from Acanthamoeba castellanii, yeast, and cow and observed virtually identical results. After tomography, they used computational docking methods to fit the known crystal structures of the Arp2/3 complex into the reconstructions. The resolutions of these reconstructions is ~2.6 nm, so individual protein subunits can be located easily and large changes in conformation within these subunits can be detected, but subtle changes in conformation cannot be resolved.

One major finding in this study is that arrangement of the Arp2 and Arp3 subunits within the complex in a branch is quite different than in the inactive soluble complex (Fig. 1). In the branch, these two subunits come together and form a short-pitch helix dimer and together contribute the first two subunits of the daughter actin filament. This observation confirms the long-held suspicions about these subunits and excludes other models that had an Arp subunit incorporated into the mother actin filament. Bringing together the Arp2 and Arp3 subunits requires a substantial rearrangement of the entire complex, and Rouiller et al. (2008) postulate that the free energy contributed by the binding of several factors (F-actin, G-actin, and activating protein) is required to drive this conformational change and overcomes the kinetic barrier to branching.

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Another particularly intriguing finding from this study is the significant alteration of the actin subunits in the mother filament that contact the Arp2/3 complex in the branch. Surprisingly, all seven subunits make some contact with the mother filament, and the total area of surface that is buried at this contact site is substantial (9,100 Å²). On the mother filament side, five actin subunits make contact with the Arp2/3 complex. Two of these subunits showed a distortion from the typical F-actin conformation in the reconstructions. In one case, the conformation was similar to the closed conformation seen in actin monomers. However, the other distorted mother filament subunit showed an open nucleotide-binding cleft reminiscent of apo (nucleotide free) Arp3. One interesting area to explore in future studies is whether these changes require activation of the complex and/or the presence of a daughter filament to occur. This information may explain the puzzling results from several studies indicating that the inactive Arp2/3 complex has a relatively low affinity (~2–4 μM) for the sides of actin filaments (Mullins et al., 1998; Gournier et al., 2001; Beltzner and Pollard, 2007).

The structure of the Arp2/3-actin branch will open many possible lines of future research. The work of Rouiller et al. (2008) provides critical information that will allow the complete elucidation of the pathway that leads to branch formation. It is known that the Arp2/3 complex, an actin filament, a nucleation-promoting protein such as SCAR or WASP, and an actin monomer must converge to form a branch, but the specific temporal sequence and the interrelationship between these binding events are incompletely understood. This question has important implications for how and where Arp2/3 branches form in cells.

Another area that this structure could potentially illuminate is the role of nucleotide hydrolysis by the Arp2 and Arp3 subunits in branch structure and debranching. Nucleotide binding and hydrolysis by both subunits are important for Arp2/3 complex catalysed branch dissociation is critical for endocytic force generation. Nat. Cell Biol. 8:826–833.

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Figure 1. Diagram showing the conformational changes in both the mother actin filament and the Arp2/3 complex upon branch formation. The yellow circle shows a comparison of the inactive, soluble Arp2/3 complex (green) and the complex in the branch (red).

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