Minireview

What initiates actin polymerization?

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Abstract

For those working on the actin cytoskeleton, a major theme of the 39th annual meeting of the American Society for Cell Biology (Washington D.C., December 11–15, 1999) was the elucidation of how actin polymerization is initiated. The emphasis was on the regulation and localization of the Arp2/3 complex, which over the last two years has been shown to stimulate actin nucleation, and on the identification of additional proteins that interact with actin and Arp2/3 in a variety of organisms.

A major breakthrough in analysing the regulation of actin polymerization has been the ability to initiate the process in vitro using purified proteins and a combination of biochemical fractionation and informed guesswork. Different laboratories have used either the bacteria Shigella and Listeria, the GTPase Cdc42, or micelles of the phospholipid phosphatidylinositol (4,5) bisphosphate (PIP2) to initiate actin polymerization in vitro. Amazingly, the cellular components required for each of these initiators to induce actin polymerization are mostly identical (Figure 1). In all cases, the Arp2/3 complex is the key central component that somehow brings together actin monomers to form a new nucleus for actin polymerization, but the precise mechanisms whereby each initiator recruits the Arp2/3 complex varies. The Arp2/3 complex can be activated in vitro by binding to the Wiskott-Aldrich syndrome protein WASP/N-WASP via the carboxy-terminal acidic region of these proteins (Tom Pollard, Salk Institute). Cdc42 can bind to full-length N-WASP and stimulate its ability to activate the Arp2/3 complex, thereby inducing actin polymerization. Shigella recruits N-WASP via its IcsA protein, whereas Listeria bypasses the requirement for WASP and binds to and activates the Arp2/3 complex directly through an acidic region of the Listeria ActA protein (Matt Welch, University of California Berkeley).

Using a biochemical fractionation approach, Rajat Rohatgi and Marc Kirschner (Harvard Medical School) reported that Cdc42- and PIP2-induced actin polymerization in vitro requires a complex of proteins that includes N-WASP, the Arp2/3 complex, and an as yet unidentified 140 kDa Cdc42-interacting protein. Polymerization can be initiated by adding purified N-WASP or the carboxy-terminal WA region of N-WASP to the other components (Figure 2). Interestingly, the amino-terminal WH1 domain of N-WASP, which has been reported to bind to PIP2, is not required for PIP2-induced actin polymerization. This implies that PIP2 does not initiate actin polymerization by binding to and activating N-WASP, but through another mechanism, possibly by activating Cdc42.

Further evidence that N-WASP is required for PIP2-activated actin nucleation was provided by John Hartwig (Harvard Medical School), who reported that in extracts of fibroblasts derived from N-WASP-deficient mice PIP2 was unable to initiate actin nucleation, whereas it could in extracts from wild-type fibroblasts. Further analysis of these fibroblasts should certainly provide important information on the role of N-WASP in actin polymerization and cell motility.

An elegant analysis of Arp2/3 localization by immunoelectron microscopy has revealed that it is localized in lamellipodia at branch points between actin filaments, reflecting its ability to bind to the sides of actin filaments. It nucleates a branching filament network in vitro, as is also found in the actin tails generated by Listeria (Gary Borisy, University of Wisconsin). This localization of the Arp2/3 complex is
consistent with it playing an important role in initiating actin polymerization in lamellipodia and by microorganisms. In contrast, the Arp2/3 complex is not localized to filopodia, consistent with the fact that actin filaments are bundled in parallel in filopodia and not in a branching network. It is likely, therefore, that a different nucleation mechanism is involved in generating filopodia, although this needs to be reconciled with published reports (Tadaomi Takanawa, University of Tokyo) showing that N-WASP mediates filopodium formation. The localization of N-WASP has not yet been analysed to the same extent as the Arp2/3 complex, mainly because of lack of good reagents, but it would be interesting to determine where it localizes by immunoelectron microscopy.

It is quite probable that N-WASP/WASP is not responsible for recruiting the Arp2/3 complex to lamellipodia, and thus the search is on for other proteins that interact with the complex. The SCAR proteins are likely candidates, but two groups at the meeting reported that the Arp2/3 complex can interact directly or indirectly with myosin I proteins (Terry Lechler and Rong Li, Harvard Medical School). Myosin I proteins differ from the classic Myosin II proteins that form muscle fibres and stress fibres, in that they have a single head domain. They also generally have an SH3 domain. In Saccharomyces cerevisiae, there are two myosin I genes: myo3 and myo5. The Myo3p and Myo5p proteins are unusual in that they have a carboxy-terminal acidic domain not found in other myosin I proteins. It turns out that they can interact directly with the Arp2/3 complex via these domains. New actin assembly can be visualized in perinuclearized S. cerevisiae by observing incorporation of rhodamine-labelled actin into actin patches. A combination of fractionation of S. cerevisiae components and genetic analysis had previously shown that Cdc42, the Arp2/3 complex and Bee1 (the S. cerevisiae homologue of WASP) are all required for actin patch formation.

Subsequent analysis of the domains of Bee1 required to initiate actin patch assembly when added back to bee1-deficient yeast showed, surprisingly, that the WA domain of Bee1 is not required for yeast to grow. The proline-rich central domain of Bee1 was able to interact with the SH3 domains of Myo3 and Myo5, however, which can in turn interact via the carboxy-terminal acidic domain with the Arp2/3 complex. In cells lacking both myo3 and myo5, there is no actin patch assembly, and if the acidic domains of Bee1, Myo3 and Myo5 are deleted, the yeast are non-viable and there is no actin assembly. Further indication that myosin activity is important for actin patch formation is provided by the observation that 2,3-butanedione 2-monoxime, a myosin ATPase inhibitor, prevents actin patch formation.

Interestingly, it has previously been shown (Ekkehard Leberer, National Research Council Biotechnology Research Institute, Montreal) that Myo3 and Myo5 are regulated by phosphorylation in their actin-binding ATPase head domain, and that they are phosphorylated by the S. cerevisiae Cdc42/Rac-interacting binding (CRIB) domain; a polyproline-rich region that can interact with SH3 domains in a variety of proteins and with profilin; a WH2 domain which interacts with actin (there are two WH2 domains in N-WASP); and an acidic carboxy-terminal domain which interacts with the Arp2/3 complex.
p21-activating kinases PAKs Ste20 and Cla4. These PAKs are targets for Cdc42, suggesting that Cdc42 could link to actin polymerization not directly via Bee1 (as is suggested by the results with WASP in mammalian cells) but indirectly via effects on myosin I proteins, which then recruit both Bee1 and the Arp2/3 complex (Figure 3a). Indeed, a constitutively active version of Myo3 or Myo5, in which the phosphorylation site for PAKs is mutated from serine to aspartate, can rescue a Cdc42ts mutant. These data provide good evidence that WASP/SCAR homologues may not always be the direct recruiters of Arp2/3 complex.

Another link between myosin I proteins and the Arp2/3 complex was provided by a combination of biochemical and genetic analysis in *Dictyostelium* (Figure 3b). John Hammer (National Institutes of Health) found that the Arp2/3 complex immunoprecipitated with myosin 1B and 1C. Two other proteins were also found in this complex: a 116 kDa protein and the actin filament capping protein CapZ. Characterization of the 116 kDa protein, p116, revealed that it is the *Dictyostelium* homologue of the Acanthamoeba protein Acan125, which was previously shown to interact with myosin I proteins. Now p116 was shown to interact via PXXP (where P is proline and X any amino acid) motifs with the SH3 domains of myosin IB and IC. The p116 protein has a carboxy-terminal acidic domain that is homologous to the acidic domain of WASP/SCAR proteins, and is predicted to mediate its interaction with the Arp2/3 complex. Interestingly, p116 co-localizes with MyoB/C and the Arp2/3 complex in micropinocytic cups on the dorsal surface of *Dictyostelium*, suggesting that it may be involved in promoting interaction between these proteins at sites of active actin polymerization. *Dictyostelium* lacking p116 grow more slowly, have a decreased rate of fluid phase endocytosis (mediated by micropinocytosis), and migrate more slowly during chemotaxis.

So what is the role of MyoB/C in regulating the Arp2/3 complex? My guess is that next year’s ASCB meeting will have lots of new information on myosin I proteins and the Arp2/3 complex derived from species such as Acanthamoeba, yeast, Dictyostelium and others, through to mammalian cells. Then we may know whether myosin I proteins act primarily as transporters of the complex into lamellipodia or into yeast actin patches and to the barbed ends of filaments, or whether they are directly involved in promoting Arp2/3-mediated actin nucleation. This is also likely to be the start of a more concerted search for other proteins that interact with and/or activate the Arp2/3 complex. Using the WASP family and yeast myosins, genome database searching for proteins with homologous Arp2/3 complex-interacting domains may well be the key to some more surprises in this field.

Figure 3
Roles of myosin I proteins in recruiting the Arp2/3 complex. (a) In *S. cerevisiae*, Myo3 and Myo5 can recruit the Arp2/3 complex directly through a carboxy-terminal acidic domain. (b) In *Dictyostelium*, the myosin I proteins MyoB and MyoC can recruit the Arp2/3 complex indirectly through p116, which also interacts with the actin filament capping protein CapZ.