Comment

The Tails of Two Myosins

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Two papers in this issue of *The Journal of Cell Biology* uncover a possible new connection between the actin-nucleating complex of proteins, the Arp2/3 complex, and the type I myosin motors. In this issue, Lechler et al. (2000) and Evangelista et al. (2000) show a direct interaction of the *S. cerevisiae* myosin I motors (Myo3p and Myo5p) with the Arp2/3 complex through an acidic COOH-terminal sequence motif. The data suggest that a large complex containing both myosin motors and actin nucleating proteins may be a functional unit for signal-induced actin assembly. Furthermore, both studies provide evidence that myosin I function is essential for assembly and maintenance of filamentous actin structures in cells. This is exciting and raises some controversy, given the recent discovery that intracellular pathogens such as *Shigella flexnerii* and *Listeria monocytogenes* do not use myosin motors to achieve actin-based motility (Loisel et al., 1999). Clearly, future research will be devoted to resolving the role of myosin I motor activity in actin-based motility in eukaryotic cells, as this may constitute a mechanistic difference between the *Listeria* and *Shigella* model systems and eukaryotic cell motility.

The Arp2/3 complex nucleates new actin filament assembly, most likely in response to signals such as the activation of receptor tyrosine kinases or receptors coupled to small GTPases of the Rho family (e.g., Rho, Rac, and Cdc42; Machesky and Insall, 1998; Svitkina and Borisy, 1999). It is named Arp2/3 because in addition to five unique polypeptides, it contains the actin-related proteins, Arp2 and Arp3. In vitro, the Arp2/3 complex cross-links actin filaments, caps the slow-growing (pointed) end of filaments (Mullins et al., 1998), and nucleates actin assembly (Mullins et al., 1998; Welch et al., 1998). This activity can be greatly stimulated by direct interaction with proteins of the WASP family (Machesky and Insall, 1999; Svitkina and Borisy, 1999). WASP family proteins are named for Wiskott-Aldrich syndrome, a fatal immune disease in humans that results from mutations in the gene encoding WASP (Thrasher et al., 1998). In *S. cerevisiae*, the single WASP family protein is called Las17p or Bee1p (Fig. 1). While the details of how the WASP family proteins activate the Arp2/3 complex are not yet known, a conserved acidic tail sequence in all WASP family members binds directly to the complex (Machesky et al., 1998; Winter et al.,

1999). This sequence motif is highly homologous to the Myo3p and Myo5p acidic tail sequences (Fig. 1), suggesting that these myosins may connect to the Arp2/3 complex in a similar fashion to WASP family proteins.

Myosin I motors have been implicated in transport of membrane vesicles in endocytosis and also in polarized cell growth and motility (Coluccio, 1997; Raposo et al., 1999). Myosin I proteins can bind to membrane phospholipids via a conserved stretch of basic sequence (Fig. 1) and to actin filaments via the motor region (Fig. 1), providing a potential link between the actin cytoskeleton and membrane vesicles or the plasma membrane (Adams and Pollard, 1989). They also contain a conserved SH3 domain (Fig. 1), which, in *Acanthamoeba*, binds to a protein called Acan125 which has homologues in *Dictyostelium* and mammals (Xu et al., 1997, 1995).

Both Lechler's and Evangelista's studies emphasize that there is functional redundancy between the acidic Arp2/3 complex binding sites of Myo3p, Myo5p, and Bee1p. This is particularly interesting, as it suggests that multiple Arp2/3 activating motifs may exist in all cells, providing a backup or alternative system for regulating actin assembly. While it is easier to picture systems that work in a linear fashion, more frequently, cells seem to use a multiply redundant or circular system where large complexes can form among proteins with multiple binding sites and many partners. Focal adhesion complexes of mammalian cells provide one example of this. This may provide flexibility, such as the ability to build large and small assemblies according to the task at hand, and/or as with the myosin I motors, it may allow several relatively weak interactions to add up into a fairly stable but dynamic assembly.

The functional redundancy also suggests that Myo3p and Myo5p can somehow promote activation of the Arp2/3 complex in a way similar to WASP family proteins. This is surprising, given the lack of a WH2 motif in Myo3p or Myo5p (Fig. 1). The WH2 motif appears to be required for WASP family proteins to activate nucleation by the Arp2/3 complex (Machesky et al., 1999). Perhaps the interaction of Myo3p and Myo5p with verprolin, which lacks an Arp2/3-binding site but has a WH2 motif (Fig. 1), supports the stimulation of actin nucleation. Mechanisms of activation of Arp2/3 complex and the importance of the WH2 motif require further study.

Lechler et al. demonstrated a requirement for myosin I motor activity in actin assembly in permeabilized cells. This comes as a surprise, given that in a reconstituted system using *Listeria monocytogenes*, actin-based motility

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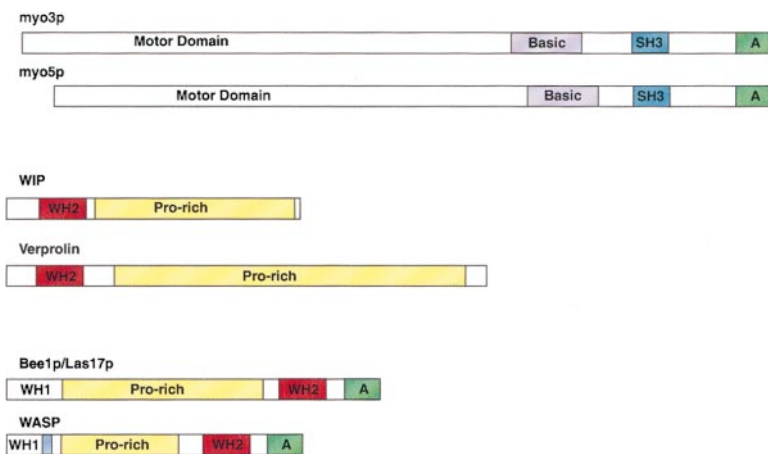
Figure 1. Schematics of the sequences of Myo3p, Myo5p, WIP, verprolin, Bee1p/Las17p, and WASP. Molecules and sequence domains/motifs are drawn roughly to scale. Myo3p and Myo5p contain basic sequences (purple) that interact with negatively charged phospholipids, and SH3 domain (yellow) and an acidic tail sequence (green) which binds to the Arp2/3 complex. WIP and verprolin contain a WH2 (WASP homology 2 motif that binds to monomeric actin, red) and are mostly proline-rich (yellow). Bee1p/Las17p and WASP contain WH1 (WASP homology 1, white), proline-rich sequences (yellow), WH2 motifs (red) and acidic tail sequences that bind to Arp2/3 complex (green). WASP also contains a Cdc42-binding motif (blue) while Bee1p/Las17p does not.

was supported by purified cofilin, Arp2/3 complex and capping protein in the absence of any myosin motor activity (Loisel et al., 1999). However, intracellular pathogens may not be perfect models for actin-based motility. The bacterial surface lacks actin-membrane interfaces, while eukaryotic cells primarily seem to polymerize actin in association with lipid membrane surfaces. Alternatively, the constitutive actin-based motility of *Listeria* may mimic a different intracellular pathway than the Cdc42p-induced actin polymerization studied by Lechler et al. and Evangelista et al. Many different signals can trigger actin assembly in cells, so there could be some pathways that use myosins and others that do not.

Myosin I motors work in clusters, giving them additive strength and perhaps processivity (Ostap and Pollard, 1996). The two studies featured here suggest that in addition to membrane binding, myosin I may be clustered via an interaction with WASP family proteins. Bee1p has a binding partner called verprolin or Vrp1p. Verprolin has sequence similarity to Bee1p in the proline-rich region and in the actin-binding WH2 sequence (Fig. 1). Verprolin also has an apparent mammalian counterpart, WIP, which binds to WASP (Fig. 1). The proline-rich sequences of both verprolin and Bee1p interact with the myosin I SH3 domain, to create several potential myosin I binding sites on the verprolin/Bee1p complex. This could, in turn, create several potential Arp2/3 complex binding sites. Altogether, a complex could form which contains from 2–21 myosin Is, 3–22 Arp2/3 complexes, and 2 actin monomers per Bee1p/verprolin complex. Of course, steric hindrance may prevent such large complexes from forming, so we await further characterization of the actual stoichiometry. While it is attractive to speculate that actin filament assembly could involve clusters of membrane-bound myosin I and WASP family proteins, we do not yet have enough information to form a complete model. Evangelista et al. and Lechler et al. both speculate that actin filament elongation may be regulated by myosin I in a similar way to microtubule motors which grasp the ends of mirotubules and allow or facilitate addition of subunits at the plus end. The myosin I clusters could stay associated with the plasma membrane and with actin filaments and push out the membrane allowing the filaments to elongate. The big-

gest conceptual problem with this model is the lack of processivity of myosin I motors due to weak binding to actin filaments (Ostap and Pollard, 1996). Myosin I spends most of its time dissociated from the actin filament, unlike kinesin motors, which spend more time associated. Due to the
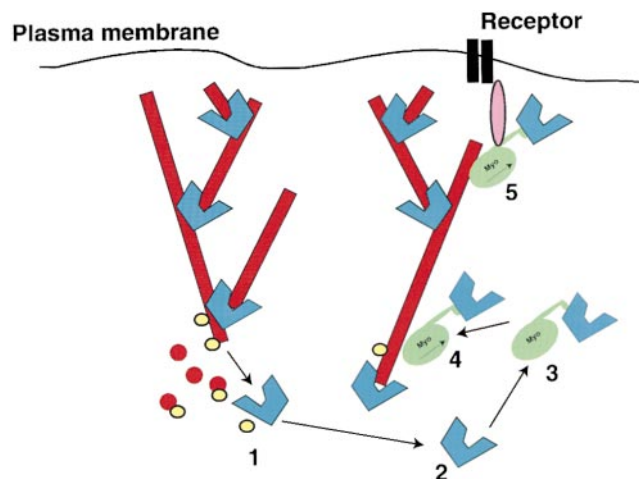

Figure 2. Myosin I may transport Arp2/3 complex to sites of actin polymerization. (1 and 2) Arp2/3 complex (blue) dissociates from an older branchpoint, allowing cofilin (yellow circles) to accelerate the disassembly of older filaments. Cofilin binds to the sides of actin filaments (red lines) and to actin monomers (red circles). (3) Myosin I clusters could then bind to the free Arp2/3 complex via the myosin I acidic tail sequence. (4) Once attached to a filament, the myosin I cluster could transport Arp2/3 complex back to the plasma membrane. (5) When the myosin I cluster arrives at the leading edge of the cell, it could dock via its SH3 domains contacting WASP family protein polyproline sequences. This could provide activated Arp2/3 complex in zones of nucleation of new actin. Myosin I clusters could then hand the Arp2/3 complex over to a WASP family protein and dissociate, or remain bound in a large complex (see Discussion in Lechler et al. and Evangelista et al.). Although there are several potential binding sites on WASP family proteins and verprolin for myosin I, and myosin I must work in clusters to be processive, only one myosin I per Arp2/3 complex has been drawn here for simplicity.

kinetics of association and dissociation of myosin I, Ostap and Pollard (1996) predicted that clusters of >20 myosin I molecules would be needed for processive motility. The reason for an apparent requirement for myosin I motor activity in actin assembly in the reconstituted system of Lechler et al., thus, remains a bit of a mystery.

Another model suggests that myosin I could transport the nucleation machinery to the barbed ends (fast-growing) of filaments. This could include transport back to the plasma membrane of Arp2/3 complexes that dissociate from the actin filament network following depolymerization (Fig. 2). This model also has the problem with processivity described above. It would also require either that clusters of myosin I travel in a branched network of actin filaments, or that some long relatively unbranched filaments also exist in lamellipodial zones. It is not obvious how the WASP family proteins are important in this model, unless they also require transport or unless the myosin I uses its SH3 domain to dock on a WASP family protein when it reaches the plasma membrane. Clearly, there are several interesting possibilities, which future studies will no doubt resolve.

Given the new information raised by these two studies, many questions arise. How does the mammalian system, where no known myosin I protein contains an acidic tail, compare to the *S. cerevisiae* system? Does the myosin I SH3 domain connect mammalian myosin Is to the Arp2/3 complex via a WASP family protein or WIP? How many redundant Arp2/3 complex binding sequences exist in eukaryotic cells? The next step may be to look for the proposed clusters of myosin I proteins to test whether these tentative models have a solid physiological grounding. Zot et al. (1992) showed that myosin I motors could move actin filaments along lipid substrates in vitro, so it should be possible to test whether myosin I can transport Arp2/3 complex along actin filaments.

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