

Extending the Arp2/3 complex and its regulation beyond the leading edge

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Two studies characterizing *Drosophila* Arp2/3 complex and Scar mutants demonstrate that assembly of some actin structures in nonmotile cells of multicellular organisms utilizes the same proteins as are important for actin assembly in motile cells. These studies also show that assembly of other actin structures is independent of these proteins, suggesting that alternative mechanisms also exist.

Studies of the mechanisms of actin assembly *in vivo* have centered on the leading edge of motile cells and much is now known about actin organization in this region. Until recently, however, the mechanism by which new filaments are initiated has remained unclear. Studies of the Arp2/3 complex and its activators, particularly the WASp/Scar family proteins, have led to a new level of understanding of nucleation and branched network formation at the leading edge (for review see Higgs and Pollard, 2001). However, the role of the Arp2/3 complex and its regulators in the assembly, organization, and maintenance of a variety of actin structures in the many cell types of a multicellular organism has not yet been explored. In two papers (Hudson and Cooley, 2002; Zallen et al., 2002, this issue), the role of Arp 2/3 and its activator, Scar, are investigated using mutants in *Drosophila*. These are the first mutants in these proteins available in a multicellular organism. These studies make it clear that some cell type-specific actin structures require Arp2/3 for assembly, whereas other structures within the same cells do not. In addition, this work highlights the importance of Scar as the primary Arp2/3 complex activator used in most cells in *Drosophila*. These studies are a good beginning toward an understanding of the complexities of assembling actin structures of varied morphologies in many different cellular contexts.

The recent explosion in our understanding of the process of actin network assembly began with discovery of the Arp2/3 complex (Machesky et al., 1994), a complex of seven proteins that is the only known nucleator of new actin filaments (Pollard et al., 2000). In addition to having nucleation activity,

the complex binds to the sides of preexisting actin filaments to mediate the formation of the dynamic branched networks seen at the leading edge of motile cells. The dendritic nucleation model (Pollard et al., 2001; Fig. 1) summarizes the current thinking about how this complex works at the leading edge. The analysis of *Drosophila* Scar and Arp2/3 complex mutants advances our understanding to include some actin structures of specialized function in nonmotile differentiated cells.

The two papers examine oogenesis and early embryonic development in some depth, with limited analysis of other developmental stages. For the purposes of this discussion, the studies of oogenesis are most informative. During oogenesis, loss of Arp3 (one of the actin-related proteins) or Arpc1 (also called p40 or SOP2) leads to defects in the expansion of ring canals (Hudson and Cooley, 2002). Ring canals are actin-lined cytoplasmic bridges that link the cells of the egg chamber and provide channels through which macromolecules are transported into the oocyte. This result demonstrates that the Arp2/3 complex regulates actin structures not involved in motility. During this stage of oogenesis in wild-type flies, the circumference of the actin ring increases without an increase in filament numbers. The actin filaments in ring canals are organized in loosely packed antiparallel bundles (Tilney et al., 1996), an organization very different from the branched network at the leading edge of moving cells. There are two potential ways the Arp2/3 complex could function in these parallel bundles. Arp 2/3 may nucleate and bind to the sides of preexisting filaments, as it does at the leading edge. These branches then may be quickly lost, and bundling proteins (such as filamin, kelch, and cheerio) may stabilize the parallel organization of the new filaments. Alternatively, only the nucleation activity and not the side binding activity of the complex may be required for polymerization in this filament array. Reconstitution experiments show that Arp3 and Arpc1 subunits are important for nucleation, whereas other subunits are more important for side binding (Gournier et al., 2001). Side binding stimulates Arp2/3 complex nucleation *in vitro* (Machesky et al., 1999, Zalevsky et al., 2001), so these activities are thought to be coupled. Whether this is always true *in vivo* remains to be tested. Understanding Arp2/3 complex participation in ring canal expansion requires reconciliation of the need for nucleation of new filaments with the observation that, at this stage, the number of actin filaments does not in-

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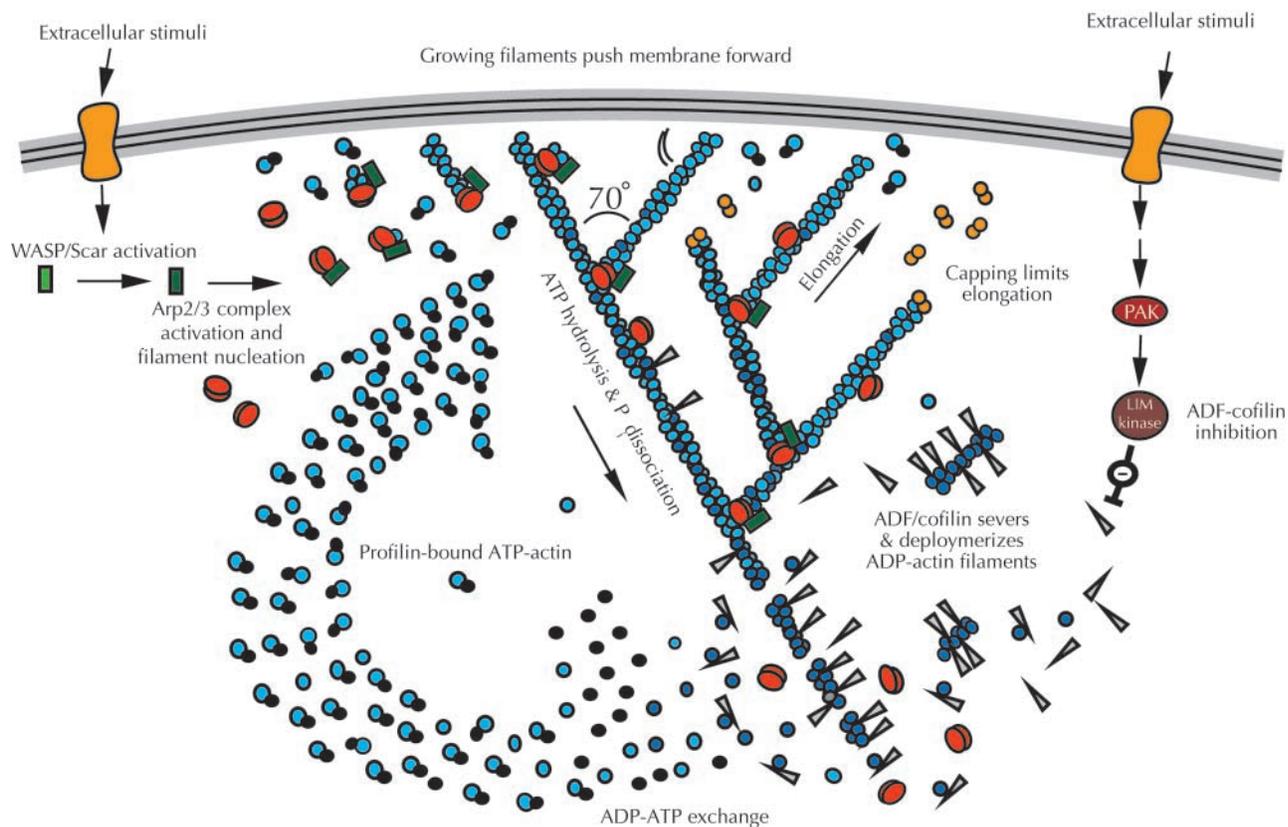


Figure 1. The dendritic nucleation model of actin dynamics at the leading edge of motile cells. Actin (blue circles) at the leading edge of migrating cells is organized in a branched network (blue polymers), with the fast growing, so-called barbed ends of the filaments pointing toward the membrane. Polymerization occurs at the free barbed ends, and as it does, the force generated by the rapid growth of the actin network leads to protrusion of the membrane. The initiation of new filaments relies on Arp2/3 complex (red circles), activated by a protein such as SCAR or WASp (green rectangles). Scar, WASp, or other activators (not depicted) respond to a variety of signals (yellow oblong on the left). The branching morphology is generated by the binding of Arp2/3 complex to the sides of preexisting filaments. The other proteins and activities depicted are not discussed in this review. (Adapted with permission from Pollard et al., 2001.)

crease (Tilney et al., 1996). Why Arp2/3 mediated nucleation is important at this step, but not during earlier stages when filament number is increasing, is not obvious.

The data of Hudson and Cooley (2002) also support the idea that the Arp 2/3 complex is dispensable for assembly of some actin structures in the same cells. Initial formation of cytoplasmic bridges and their morphogenesis into early ring canals is unaffected, as is cell division, using cytokinetic contractile rings. In addition, the more ordered actin bundles that form in nurse cells late in development are unaffected. General cell morphology, presumably dependent on cortical actin arrays, is also normal (at least until very late stages).

At other times in development, a similar picture emerges. Mutants display effects on actin bundles in bristles (only minor defects; Hudson and Cooley, 2002), in the early embryo (major defects in actin caps and furrows), in the central nervous system (problems in axon morphology), and in eye development (abnormal cell morphology; Zallen et al., 2002). Many other cell types however are not obviously affected. This implies that as yet undiscovered nucleators may exist that mediate actin assembly in cases where the Arp2/3 complex is not required. Alternatively or in addition, other mechanisms, such as uncapping or filament severing, might be important for these other actin arrays.

When similar cell types and times in development are examined in SCAR mutants (Zallen et al., 2002), the defects observed are identical to those seen in loss of Arp2/3 complex mutants. This in itself is significant in that it demonstrates that Arp2/3 complex is Scar's only major target.

In contrast to the extensive defects seen in Scar mutants, WASp mutants show no defects during oogenesis, early embryonic development, or CNS development (although WASp may work redundantly with Scar in this context; Ben-Yaacov et al., 2001; Zallen et al., 2002). This implies that Scar is the more ubiquitous regulator of Arp2/3 complex *in vivo*, even though WASp is a more potent activator *in vitro* (Zalevsky et al., 2001). Scar's wider role *in vivo* suggests that extremely fast polymerization may not be critical in many situations. Or perhaps there are mechanisms that enhance Scar-mediated Arp2/3 complex activation *in vivo* that have not been mimicked *in vitro*.

In direct comparisons of WASp and Scar function during oogenesis, early embryonic development, CNS development, and eye development, there is little overlap in phenotypes (Ben-Yaacov et al., 2001; Zallen et al., 2002). This demonstrates that these two activators independently regulate assembly of actin structures in distinct cell types and contexts. The Arp2/3 mutant defects appear to

be the sum of the defects seen in WASp and Scar mutants, indicating that other Arp2/3 activators (Pollard et al., 2001), such as cortactin or pan1/Eps15, do not have prominent roles in vivo. However, not all developmental times and cell types have been assayed in this work. Roles for other activators may become apparent as other actin-based morphologies and processes are investigated using these mutants.

Another interesting observation is that complete loss of function of either Arp3 or Arpc1 produces identical phenotypes (Hudson and Cooley, 2002). In yeast, some data suggest that Arpc1 may have functions outside the complex (Winter et al., 1999), but there is no support for this idea from the data presented in this paper. The analysis of oogenesis is sufficiently detailed to predict that, if there were differences, they might have been detected. However, other phenotypes are not analyzed in detail in both mutants, so how widely this conclusion can be applied is unclear.

These papers raise new questions and highlight areas where discoveries are likely. The mechanisms that regulate assembly of the many actin structures not affected in these mutants remain unclear, and the roles of Arp 2/3 activators other than WASP or Scar in vivo are not obvious. How Arp2/3 complex-mediated actin polymerization leads to ring canal expansion has not been addressed, nor have the mechanisms by which other reported defects are generated been explored. Despite these questions, it is clear that the Arp2/3 complex, activated by Scar, is critical for generating actin structures in both nonmotile cells of multicellular organisms and in highly motile single cells.

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