

Signaling to Actin Dynamics

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THE actin cytoskeleton of a eukaryotic cell is central to locomotion, phagocytosis, contractility, shape changes, cytokinesis and maintenance of polarity. The mechanisms through which actin coordinates these different activities have been fascinating for many years, but the pace of discovery has recently been especially rapid. This review will concentrate on how cells control actin polymerization to produce the force for motility and shape changes.

It is becoming clear that the Arp2/3¹ complex, a complex of seven proteins including the actin-related proteins Arp2 and Arp3, regulates the assembly of new actin filament networks at the leading edges of cells. Proteins of the WASP (Wiskott-Aldrich Syndrome protein) family bind directly to the Arp2/3 complex and stimulate its ability to promote the nucleation of new actin filaments. Upstream of WASP-family proteins, receptor tyrosine kinases, the Rho-family GTPase Cdc42, and likely G protein-coupled receptors, receive and transmit the signals leading to WASP-Arp2/3 complex-mediated actin nucleation. Together, these ideas and discoveries suggest complete signaling pathways from outside of the cell to actin polymerization-driven cell motility.

While the Arp2/3 complex and WASP family proteins are very important, any model of actin polymerization must include many other participants, in particular Ena/VASP, gelsolin, and capping protein. Members of the Ena/VASP family colocalize with the Arp2/3 complex at the leading edges of cells and catalyze the elongation of newly formed filaments. Capping protein and gelsolin regulate the growth of actin filaments by terminating elongation. They also mediate associations between actin and the plasma membrane, and may promote or permit filament elongation under the control of membrane phospholipids.

Actin Polymerization in Cell-free Systems and the Role of Cdc42

Recently, several groups have developed systems which allow reconstitution of actin polymerization in vitro (Ma et al.,

1998a; Moreau and Way, 1998; Zigmond et al., 1997). Two groups have used lysates from *Xenopus* eggs, in each case activated by the addition of Cdc42. Synthetic lipid vesicles containing phosphatidylinositol 4,5 bisphosphate (PIP₂), when added to these lysates, generate rocket-like structures containing tails of polymerized actin (Ma et al., 1998a; Moreau and Way, 1998; Fig. 1 A). Both Cdc42 and PIP₂ are necessary for actin tails, which suggests that these signaling molecules play important roles in vivo. However, Zigmond et al. showed that Cdc42 could induce actin polymerization in a similar cell free extract, but apparently independently of PIP₂ (Zigmond et al., 1997). This actin, rather than being organized into rocket-shaped motile structures, forms a fine meshwork of filaments. The Arp2/3 complex is required for Cdc42-induced actin polymerization in *Acanthamoeba* extracts (Mullins and Pollard, 1999), and for actin rocket formation on PIP₂ vesicles (Ma et al., 1998b). In addition, other unknown factors must be required, as actin polymerization cannot yet be reconstituted from purified proteins. However, taken together these experiments have set the stage for future understanding of a complete signaling pathway to the actin cytoskeleton at the molecular level.

The work described in the remainder of this review points to the likely essential components of a fully reconstituted actin-based motility system. These are the Arp2/3 complex, a system to recruit and activate Arp2/3, an Ena/VASP based catalyzer of elongation, and a capping/depolymerizing system to regulate dynamics.

The Arp2/3 Complex and the WASP Family

Interest in the Arp2/3 complex has been intense since it proved essential for reconstitution of both in vitro actin-based motility and actin polymerization on the surface of the intracellular parasite *Listeria monocytogenes* (Welch et al., 1997b; May et al., 1999; Fig. 1 C). The Arp2/3 complex was first discovered in *Acanthamoeba* as a component of the actin cortex (Machesky et al., 1994). It localizes to the leading edge of a variety of cells (Fig. 1 E; Machesky et al., 1997; Mullins et al., 1997; Welch et al., 1997a). In vitro, it caps the slow-growing (pointed) end of actin filaments (Mullins et al., 1998), making it uniquely able to stabilize free fast-growing (barbed) ends, at which nearly all actin polymerization in vivo appears to occur. This pointed end capping has been postulated to protect actin branches from the actin depolymerizing activity of cofilin (Svitkina and Borisy, 1999), but at least in vitro, this does not appear

1. *Abbreviations used in this paper:* Arp, actin-related protein; LPA, lysophosphatidic acid; PIP₂, phosphatidylinositol 4,5 bisphosphate; RTK, receptor tyrosine kinase; WASP, Wiskott-Aldrich syndrome protein.

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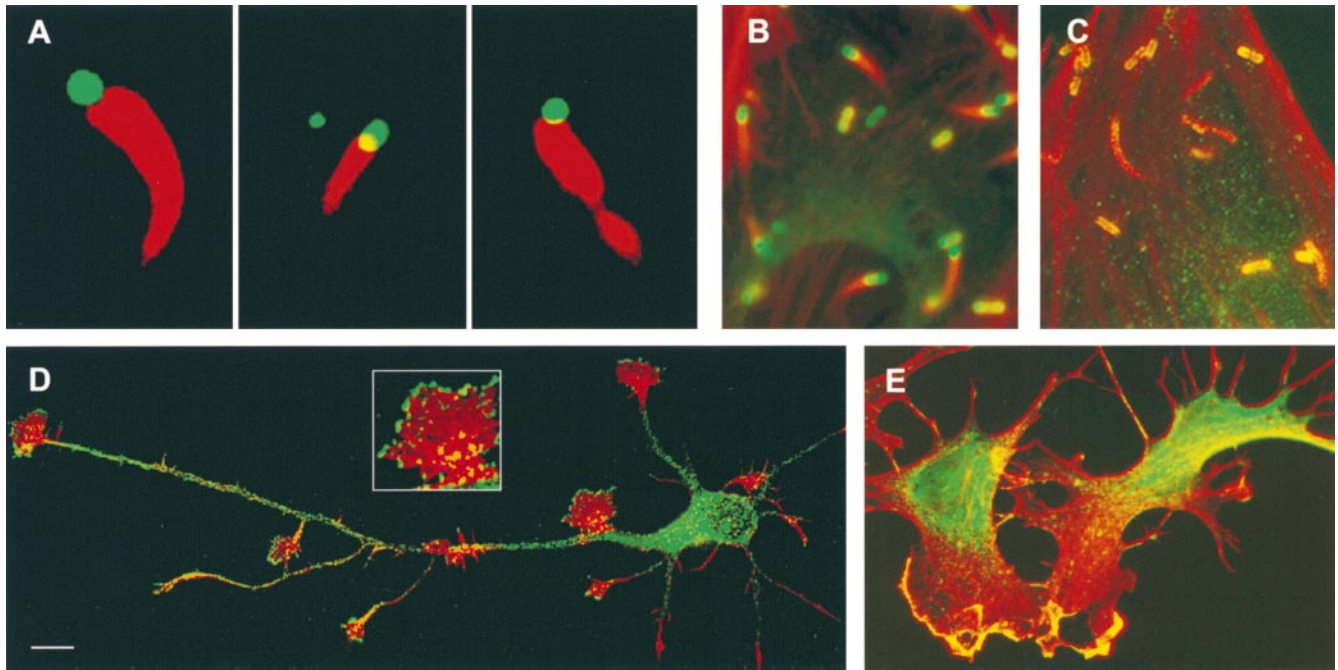


Figure 1. Localization of actin, Arp2/3 complex, and Ena/VASP proteins. (A) Actin rockets on synthetic PIP₂-containing lipid vesicles in *Xenopus* extracts. The lipid vesicle is shown in green and rhodamine-labeled actin is shown in red. (B) Costaining of filamentous actin (red) and VASP (green) on a cell infected with *Listeria monocytogenes*. VASP is clearly localized around the bacteria and at the tail-bacterium interface. (C) Costaining of filamentous actin (red) and the p41-Arc subunit of the Arp2/3 complex (green) in cells infected with *Listeria monocytogenes*. p41-Arc colocalizes along the length of the *Listeria* tails with filamentous actin. (D) Embryonic mouse hippocampal neuron in culture stained for filamentous actin (red) and Mena (green). The inset shows a close-up of a growth cone, with Mena at the tips of filopodia. (E) Swiss 3T3 fibroblasts costained for filamentous actin (red) and the p34-Arc subunit of the Arp2/3 complex (green). Photo credits: (A) Le Ma and Marc Kirschner; (B and C) Uwe Carl, Antonio Sechi, and Juergen Wehland; (D) Lorene Lanier and Frank Gertler.

to be the case (Ressad et al., 1999). The Arp2/3 complex also binds to the sides of filaments and cross-links them or forms branches (Mullins et al., 1998). Additionally, the Arp2/3 complex weakly promotes the nucleation of new actin filaments (Mullins et al., 1998; Welch et al., 1998). These data, together with electron microscopic evidence for branched actin filaments in the lamellipodia of migrating cells (Svitkina et al., 1997; Bailly et al., 1999; Svitkina and Borisy, 1999), have led Mullins et al. to propose the dendritic nucleation model for actin polymerization (Mullins et al., 1998). In this model, Arp2/3 complex is recruited to the leading edge of the cell and activated to form new actin nuclei which branch off existing filaments.

One of the most important questions about the role of Arp2/3 complex in lamellipodia concerns how it becomes localized and activated at sites of new actin polymerization. A family of candidates has now been found, including WASP, its more widely expressed homologue N-WASP and a related protein group, the Scars. These proteins bind directly to the Arp2/3 complex and regulate its behavior in cells (Machesky and Insall, 1998). This connection between a family of signaling proteins and the Arp2/3 complex suggests a pathway through which multiple signaling cascades could activate actin polymerization. WASP and N-WASP bind to receptor tyrosine kinases such as the PDGF and EGF receptors, via adapter molecules such as Nck and Grb2. WASP and N-WASP also bind to Cdc42,

and are therefore implicated in actin cytoskeletal reorganization downstream of Cdc42 (reviewed in Ramesh et al., 1999). Scar proteins appear to be regulated differently. Scar was discovered in *Dictyostelium*, as a suppressor of a deletion in the cyclic AMP receptor cAR2 (Bear et al., 1998), which suggests that Scar might be involved in pathways through serpentine receptors and heterotrimeric G-proteins.

Members of the WASP family are composed of at least one domain and several motifs which connect them to upstream signaling and downstream cytoskeletal ligands. Two WASP proteins (WASP and N-WASP; Derry et al., 1994; Miki et al., 1996) and four mammalian Scar homologues (Bear et al., 1998) have been described, one of which (Scar1) has also been called WAVE (Miki et al., 1998). Fig. 2 illustrates the organization of WASP family proteins, as compared with the Ena/VASP proteins, which are distant relatives. The amino-terminal portion of these proteins comprises a conserved region called the EVH1 domain. Although this domain shares a similar structure with PH and PDZ domains, it apparently performs a unique function, interacting with proline-rich target sequences (Prehoda et al., 1999). For Ena/VASP proteins, the polyproline-containing ligands in cells include vinculin and zyxin, while for WASP and N-WASP, the most likely candidate is the WASP interacting protein WIP (Ramesh et al., 1997, 1999). Previous identification of a PH domain in WASP and N-WASP was based on very weak homology

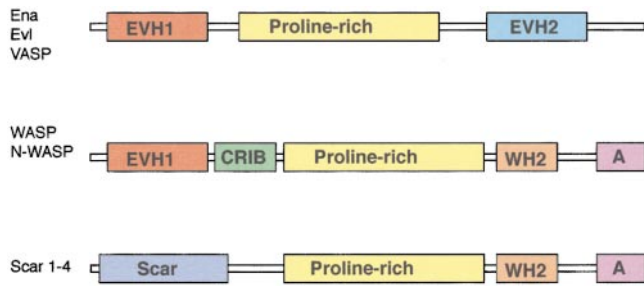


Figure 2. Domain/motif organization of WASP and Ena/VASP proteins. WASP and N-WASP contain an EVH1 domain, which is a relative to the PTB and PH domain and is likely to interact specifically with polyproline containing ligands (Prehoda et al., 1999). All proteins in this extended family also contain various proline-rich sequences that can interact with profilin and with SH3 domain-containing proteins. Ena/VASP proteins contain a COOH-terminal EVH2 sequence. WASP and N-WASP contain a CRIB motif, which confers binding to the small GTPase Cdc42 and more weakly to Rac. All WASP-family members contain a WH2 motif which interacts with monomeric actin and a COOH-terminal A motif which binds to the p21-Arc subunit of the Arp2/3 complex.

and is most likely coincidental (Miki et al., 1996; Insall and Machesky, 1999; Prehoda et al., 1999). However, N-WASP interacts with phospholipids such as PIP_2 (Miki et al., 1996).

Just COOH-terminal to the EVH1 domain in WASP and N-WASP is a CRIB motif, which confers interaction with small the GTPase Cdc42 (Burbelo et al., 1995; Aspenstrom et al., 1996; Symons et al., 1996). The central portions of both WASP-family and Ena/VASP proteins contain proline-rich sequences which can bind to the actin-binding protein profilin (Suetsugu et al., 1998) or SH3-containing proteins. The COOH terminus of WASP family proteins is made up of two regions. One is an actin-binding motif, known as a WH2 motif or verprolin homology motif (Symons et al., 1996; Machesky and Insall, 1998; Miki and Takenawa, 1998). The second is the A motif, which includes a cluster of acidic residues and mediates binding to the Arp2/3 complex (Machesky and Insall, 1998).

Control of Arp2/3 Activity by Scars, WASPs, and Signaling

Since WASP-family proteins bind to the Arp2/3 complex, the next question to ask was whether this interaction caused activation of the nucleation activity of the Arp2/3 complex. Indeed, Scar1 (Machesky et al., 1999), N-WASP (Rohatgi et al., 1999), WASP (Yarar et al., 1999), and the yeast WASP homologue Las17p/Bee1p (Winter et al., 1999) all greatly activate the ability of the Arp2/3 complex to nucleate actin filaments. In addition, Cdc42 and PIP_2 cooperate with N-WASP to activate Arp2/3 complex in actin nucleation assays (Rohatgi et al., 1999). This suggests a signaling pathway from inositol phospholipids to Cdc42 and WASP/N-WASP, which accords with the results from cell-free systems described earlier. The enhancement of nucleation by Scar1 can be further potentiated by adding actin filaments to the reaction (Machesky et al., 1999). It

appears that the binding of Arp2/3 complex to the sides of actin filaments further promotes its activation by Scar1, leading to dendritic nucleation. The presence of actin filaments may therefore act as a kind of positive feedback for the polymerization of more actin at the leading edges of cells.

Previous studies suggested that WASP and N-WASP were actin depolymerizing proteins, and that their effects on the cytoskeleton were mediated by direct interactions with profilin or actin (Miki et al., 1996, 1997; Symons et al., 1996; Suetsugu et al., 1998). However, in all studies published so far, stoichiometric quantities of N-WASP (Suetsugu et al., 1998) or Scar1 (Machesky and Insall, 1998) to actin monomer are required to affect actin filament assembly. This suggests that WASP family proteins do not disassemble intact actin filaments, as proteins such as cofilin do, but rather that they associate with actin monomers. Profilin sequesters actin monomers and inhibits spontaneous actin nucleation in a reconstituted system containing purified Scar1 and Arp2/3 complex (Machesky et al., 1999) but does not enhance either actin nucleation or elongation. It may be that profilin has a different effect in living cells or extracts, where the nucleotide bound to actin must exchange during recycling. However, at present we cannot conclude that profilin binding to WASP family proteins has an important role in cytoskeletal reorganization.

Ena/VASP Proteins Are Important Regulators of Actin Assembly

In addition to Arp2/3 complex-mediated actin nucleation, both mammalian cells and *Listeria* appear to use Ena/VASP proteins to promote actin filament assembly. The *enabled* gene was originally identified as a suppressor of Abl-dependent phenotypes in *Drosophila* axon guidance (Gertler et al., 1995), and VASP was first identified as a substrate of cyclic nucleotide-dependent kinases (Halbrugge and Walter, 1989). Ena/VASP proteins are related to WASP-family proteins in that they contain a conserved amino-terminal domain called EVH1 (Ena/VASP homology; Fig. 2; Symons et al., 1996; Prehoda et al., 1999). Members of both families also contain proline-rich sequences that bind to profilin and to SH3 domains (Fig. 2).

Mice with a VASP gene disruption exhibit defects in the cAMP- and cGMP-mediated inhibition of platelet aggregation (Aszodi et al., 1999). Mena null mice have a far stronger phenotype, including severe brain and neural defects, which indicates an essential role for Mena in the developing nervous system (Lanier et al., 1999). Additionally, Mena null mice containing a heterozygous profilin deletion display a synthetic phenotype, dying in utero just before birth. Dissected embryos have defects in neurulation, indicating a crucial role for Mena, in conjunction with profilin, during neuron growth and pathfinding (Lanier et al., 1999). In neurons, Mena is concentrated in the tips of growth cone filopodia, in front of the bulk of polymerized actin, suggesting a role in the organization of actin polymerization (Lanier et al., 1999) and Fig. 1 D. In *Listeria monocytogenes*, Ena/VASP proteins localize to the bacterium-tail interface and are essential for *Listeria* motility in extracts (Laurent et al., 1999; Fig. 1 B). Current thinking is that Ena/VASP proteins recruit actin monomers via their

interaction with profilin and catalyze filament elongation via their interactions with monomers and filaments (Laurent et al., 1999). The profilin interaction may also serve to accelerate exchange of ADP for ATP on actin monomers that are recycling off older filament pointed ends (Fig. 3).

Gelsolin and Capping Protein

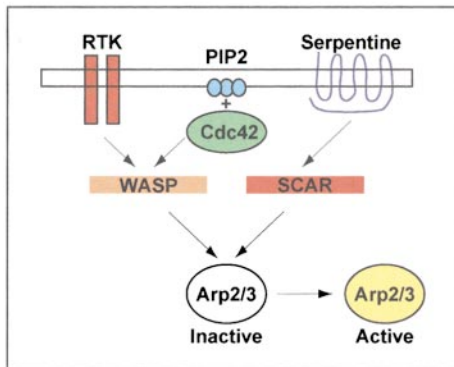
Gelsolin and capping protein have also been linked to cell signaling through polyphosphoinositides and small GTPases. Both proteins bind to the barbed ends of actin filaments and thus block filament elongation. As most barbed ends in living cells appear to be capped by these two proteins, removal of the cap in response to signals could trigger rapid, extensive actin polymerization. In human platelets, PIP₂ synthesis induced by thrombin or Rac triggers the loss of gelsolin caps from barbed ends, and subsequent rapid actin polymerization (Hartwig et al., 1995). Transgenic mouse cells do not require gelsolin to move or to polymerize actin, but show various aberrations in actin-based motility without it (Witke et al., 1995). Platelets from knockout mice show defects in aggregation and generate fewer nucleation sites upon activation (Witke et al., 1995). Similarly, gelsolin null fibroblasts show slower motility and reduced formation of lamellipodia in response to stimuli (Azuma et al., 1998).

Capping protein also appears to have a phosphoinositide-dependent role in dynamic actin remodelling. It disso-

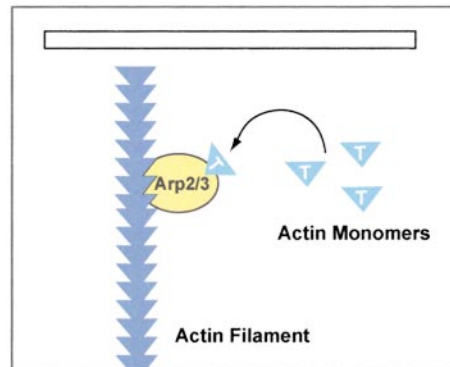
ciates from the barbed ends of actin filaments in response to PIP₂ in vitro, promoting rapid polymerization under physiological conditions (Haus et al., 1991; Heiss and Cooper, 1991; Barkalow et al., 1996; Schafer et al., 1996; DiNubile and Huang, 1997). Capping protein increases its association with actin filaments when actin polymerization is stimulated, suggesting that it terminates actin polymerization by blocking free barbed ends (Eddy et al., 1997). However, motility in *Dictyostelium* is proportional to the expression levels of capping protein, perhaps indicating a more positive role (Hug et al., 1995). In mammalian cells, GFP-capping protein localizes to regions of dynamic actin turnover, such as lamellipodia and actin spots (Schafer et al., 1998). Expression of active phosphatidylinositol 5-kinase type-Ia, an enzyme that synthesizes PIP₂, enhances the lifetime and motility of these spots, supporting a role for polyphosphoinositides in the regulation of capping protein (Schafer et al., 1998).

While it seems clear that PIP₂ and other related phospholipids have an important role in the regulation of cytoskeletal proteins, we still have a lot to learn about the details. Lysophosphatidic acid (LPA) can mediate the dissociation of gelsolin from actin and modulate the severing activity of gelsolin family members (Meerschaert et al., 1998), suggesting that some in vitro effects of PIP₂ may be regulated by LPA in living cells, maybe in concert with PIP₂. Furthermore, since more than twenty proteins are reported in the literature to be regulated by PIP₂, it seems

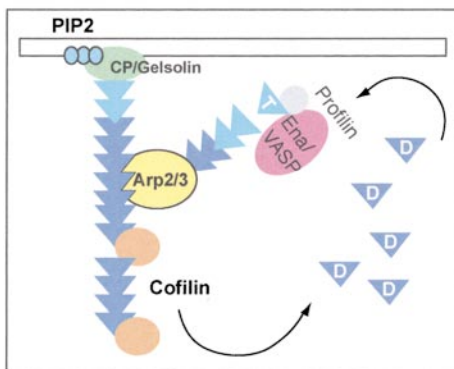
1. Localization and Activation



2. Actin Nucleation and Branching



3. Regulated Treadmilling



4. Depolymerization to Branchpoint and Release of Arp2/3 Complex

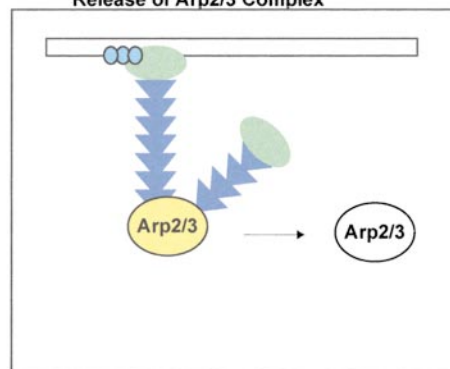


Figure 3. Regulated treadmilling model for actin dynamics. (1) Various signals, such as receptor tyrosine kinase activation, PIP₂ synthesis or serpentine receptor activation may trigger localization and/or activation of WASP/Scar proteins. WASP/Scar proteins in turn activate and localize the Arp2/3 complex to nucleate actin filaments near the plasma membrane. (2) Arp2/3 complex binds to the sides of actin filaments and rapidly nucleates branches. (3) The growing barbed ends of the filaments are dynamically regulated by capping protein and gelsolin under the control of membrane phospholipids. Ena/VASP proteins catalyze the elongation of newly nucleated filaments by shuttling activated (ATP-bound) actin monomers onto the growing (barbed) end. The shrinking (pointed) end of the filaments is depolymerizing with the help of cofilin to sever and dissociate monomers. (4) If the filaments depolymerize

down to a branchpoint, Arp2/3 complex may fall off and be recycled to participate in nucleation. Blue triangles represent actin monomers, with the light blue T monomers being ATP-bound and the darker blue D monomers being ADP-bound. RTK represents receptor tyrosine kinase, PIP₂ represents phosphatidylinositol 4,5 bisphosphate.

over-simplistic to assume that a simple rise or dip in overall PIP₂ levels could be directly controlling a process as complex as actin assembly. Several hypothetical refinements are possible. PIP₂ could exist in separate pools in vivo, which are independently localized and regulated. Alternatively, in living cells other molecules could actually mediate some of the effects caused by PIP₂ in vitro.

Regulated Treadmilling Model for Actin Dynamics

The profusion of recent data suggest a regulated treadmilling model for actin dynamics in motility (Fig. 3). This simplified model attempts to incorporate both new and old ideas in the field, including the attractive array treadmilling proposed by Svitkina and Borisy (Svitkina and Borisy, 1999). In this model, the Arp2/3 complex becomes activated through one of the WASP family proteins (which one would depend on the nature of the signal eliciting the actin polymerization). Activated Arp2/3 complex binds to the sides of actin filaments and nucleates new branches with free barbed ends. Once these dendritic structures assemble, their elongation can be controlled by capping proteins, which dynamically associate and dissociate with the barbed ends of the filaments. The relative rates of capping and uncapping can be controlled by signaling intermediates such as plasma membrane phosphoinositides. Additionally, the rate at which new filaments elongate can be accelerated by regulated association with Ena/VASP proteins and profilin. Established filaments are severed and depolymerized by cofilin, which acts more rapidly on filaments in which the monomers' ATP has been hydrolyzed to ADP (Maciver, 1998). Finally, when the filament depolymerizes all the way to the branchpoint, the Arp2/3 complex falls off and may be recycled in new filaments (Fig. 3).

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