Regulation of Actin Polymerization by Arp2/3 Complex and WASp/Scar Proteins*

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Actin polymerization is required for many types of cell motility, such as chemotaxis, nerve growth cone movement, cell spreading, and platelet activation (reviewed in Ref. 1). In the lamellipodia that push forward the leading edge of motile cells, polymerizing filaments form a meshwork consisting of “Y branches” with the pointed end of one filament attached to the side of another filament (2). This meshwork presumably may provide a rigid body against which polymerization can drive membrane protrusion (3).

A major unanswered question is how cells integrate signals coming through a variety of pathways to control when and where actin polymerizes. The filaments grow from a huge pool of un polymerized actin maintained by monomer-binding proteins at a concentration approximately 1000-fold higher than required for spontaneous polymerization of actin (reviewed in Ref. 4). The monomer-binding protein profilin biases the direction of filament elongation, allowing growth at the fast growing barbed end but not the slow growing pointed end (reviewed in Ref. 4). In cells capping proteins block the barbed end of most filaments, so some mechanism is required to start new filaments (5).

Cells might trigger actin polymerization in three ways: 1) de novo nucleation of filaments from monomeric actin; 2) severing existing filaments to create uncapped barbed ends; and 3) uncap ping existing barbed ends. There is evidence for each of these mechanisms in various cellular processes, but new filaments are often created during cell motility (6), placing emphasis on mechanisms 1 and 2.

Although activation of de novo nucleation by cell stimulation has long been an attractive model (7), no barbed end nucleating factors were known until it was discovered that Arp2/3 complex promotes actin nucleation, creating filaments that grow at their barbed ends (8). Because nucleation is rate-limiting in actin polymerization and strongly suppressed by monomer-binding proteins, Arp2/3 complex may be a key mediator of actin polymerization in cells. Arp2/3 complex also cross-links actin filaments end-to-side, indistinguishable from the Y branches at the leading edge (8).

Based on these biochemical activities, Mullins et al. (8) proposed the dendritic nuclei model, whereby Arp2/3 complex both creates new filaments and cross-links them into a branching meshwork. Cellular observations support this model. Arp2/3 complex is concentrated at the leading edge of motile cells (9–13), specifically at the junctions of the Y branches (12, 13). It exists in all eukaryotes examined, and ablation of Arp2/3 complex subunits in Saccharomyces cerevisiae and Schizosaccharomyces pombe is lethal or severely debilitating (14–21).

The next breakthrough was the discovery that ActA, a cell surface protein from the pathogenic bacterium, Listeria monocytogenes, stimulates Arp2/3 complex to nucleate actin in vitro (22). Listeria uses force generated by actin polymerization to propel itself around the cytoplasm of eukaryotic cells. ActA is the only bacterial protein required to induce polymerization, but ActA cannot stimulate actin filament formation by itself (reviewed in Ref. 23). This work suggested that cellular factors might activate Arp2/3 complex to nucleate actin.

This year WASp/Scar proteins were identified as the first example of such factors (24–28). These proteins also interact with a variety of cell signaling molecules known to influence cytoskeletal dynamics, bringing us closer to forging a connection between surface receptor stimulation and actin polymerization. The rapid progress reviewed here depended upon groundwork from many laboratories. Analysis of Wiskott-Aldrich syndrome protein (WASP) and its neural homolog N-WASP revealed a binding site for Rho family GTPases and other domains that affect actin assembly in cells (29, 30). Study of GTPyS-stimulated actin polymerization in extracts of vertebrate cells (31–34), Dictyostelium (33), and Acan thamoeba (35) demonstrated that the Rho family GTPase Cdc42 mediates the effect of GTP and that Arp2/3 complex is required. Similar experiments with extracted yeast suggested that Bee1p (a WASp homolog) and Arp2/3 complex are required for actin patch assembly (20, 36, 37).

Properties of Arp2/3 Complex

The Arp2/3 complex contains one copy of each of seven strongly associated protein subunits (Fig. 1; reviewed in detail in Ref. 38). Arp2 and Arp3 are actin-related proteins. The other five subunits are novel. The complex is very abundant, approximately 2 μM in the cytoplasm of Acanthamoeba (9). Analysis of molecular models of Arp2 and Arp3 first led to the hypothesis that they form a stable dimer that binds the pointed end of actin filaments and nucleates growth in the barbed direction (9). Polymerization assays established that the complex binds the pointed end with nanomolar affinity and has modest nucleation activity (8).

The WASp/Scar Protein Family

The mammalian WASp/Scar family currently consists of five members: WASp, N-WASP, and three Scar isoforms (Fig. 2). Several lines of evidence implicate these proteins in actin polymerization. The gene encoding WASp, apparently expressed only in hematopoietic cells, is mutated in Wiskott-Aldrich syndrome, an X-linked human disease with selective defects in platelet development and lymphocytes (29). The presence of a binding motif (GBD) for activated Cdc42 and Rac hinted that WASp might regulate actin, because these Rho family GTPases influence actin dynamics and because transfection of WASp rearranges actin filaments in cultured cells (39). The WASp homologue N-WASP is expressed more widely in vertebrate cells than WASp (30) and causes filopodial formation when co-expressed with Cdc42 in cultured cells (40). Scar was discovered in Dictyostelium where disruption of its gene rescues the developmental defect caused by disruption of a cyclic AMP receptor (41). Deletion of Scar in wild type cells causes cytoskeletal defects. A mammalian homologue of Scar, termed WAVE or Scar1, might be involved in Rac-induced membrane ruffling, although it does not contain a GBD (42). No information is yet available on the functions of two other human Scar-related open reading frames (GenBank accession numbers BAA74923 and CAA18609). In S. cerevisiae, a WASp/Scar homologue, known as Las17p or Bee1p, is essential for cortical actin patch formation and for endocytosis (37, 43).

The C-terminal 65–105 amino acids of WASp/Scar proteins (Fig. 3) enhance nucleation by Arp2/3 complex (25–28). This region starts with a WASp homology 2 (WH2) motif, a 16–19-amino acid
sequence that participates in actin monomer binding (24). N-WASP has two tandem WH2 motifs. The C-terminal 15–20 residues, designated “A,” generally possess a strong negatively net charge and interact with Arp2/3 complex (24). Between these two regions are 30–40 residues of unknown functional significance. Some have suggested that this region of WASp and N-WASP contains a short sequence similar to the actin monomer-binding protein cofilin (“cofilin homology domain”), although in our opinion the sequence similarity is too limited to indicate homology (Fig. 3). Furthermore, these short sequences cannot be in the usual sense, because in ADP/PP2A they form part of a β-strand, a loop, and part of an α-helix rather than an independently folded structure (44). However, there is evidence that this region participates in binding actin monomers (45).

The C terminus of Las17p/Bee1p differs significantly from other WASp/Scar proteins. In its WH2 motif, it has two unique inserts of unknown functional significance. The region between WH2 and A motifs differs even more, with >50% of this sequence consisting of G, A, or P. Perhaps more importantly, Bee1p is the only WASp/Scar protein in which the C-terminal 20 residues have a net neutral charge (excluding the C-terminal carboxyl group, Fig. 3). These differences might explain some functional differences of Bee1p (27).

N-terminal to this nucleation-activating region, WASp and N-WASP bind an impressive list of protein ligands (Table I): the Rho family GTPases Cdc42 and Rac, WASp-interacting protein, calmodulin, Src kinases, Tec kinases, Grb2, Nck, and profilin. These proteins provide a myriad of possibilities to regulate either the activity or location of WASp or N-WASP. No interacting proteins provide a myriad of possibilities to regulate either the activity or location of WASp or N-WASP. No interacting proteins other than actin, profilin, and Arp2/3 complex have been identified.

WASp and N-WASP also have a N-terminal WASp homology 1 domain (WH1). An atomic structure of an EVH1 domain, a homolog of the WH1 domain found in adapter proteins including Ena and VASP, shows that the fold of the domain is similar to a pleckstrin homology domain (57). Ligands with the sequence FPPPBP bind EVH1 in the place of an intrinsic α-helix found in pleckstrin homology domains, which can bind PIP2. PIP2 apparently binds an N-terminal construct of N-WASP (30) and activates nucleation by full-length N-WASP (26), but more detailed work defining the binding site is needed.
Minireview: Arp2/3 Complex and WASp/Scar

Many Questions Still to Answer

Does All Leading Edge Actin Filament Nucleation Depend on Arp2/3 Complex?—Two lines of evidence suggest that Arp2/3 complex plays a major role but do not rule out other pathways. First, most of the GTP-stimulated actin polymerization in cell extracts depends on Arp2/3 complex (32, 35), as does actin patch reconstitution in yeast (20). Second, most of the filaments at the leading edge of keratocytes and fibroblasts are incorporated into 70° branching networks with Arp2/3 complex localized at the branches (13). The affinities of Arp2/3 complex for both the pointed end and side of actin filaments allow the complex to form these branched networks (8, 58). WASp/Scar proteins are not required for branching by Arp2/3 complex but may stabilize either or both associations. On the other hand, experiments with budding yeast suggest alternative pathways. Neither Arp2 nor Arp3 is essential for viability, although null strains are extremely sick and the phenotype may depend on the genetic background of the particular strain (21).

What Signaling Pathways Converge on Arp2/3 Complex via WASp/Scar Proteins?—A variety of stimuli regulates cellular actin polymerization, acting through receptor tyrosine kinase/mitogen-activated protein kinase pathways, seven helix receptors, and integrins. Details remain unclear, but an attractive hypothesis (24) is that signals from different kinds of receptors and signaling pathways converge on particular members of WASp/Scar proteins, which funnel these signals through Arp2/3 complex as a final common pathway to actin filament formation. Any of these signals could, in principle, affect either the activity of Arp2/3 complex or its localization in cells.

The only demonstrated activators for any WASp/Scar protein are Cdc42 and PIP2. Both activate N-WASP and Arp2/3 complex to nucleate actin filaments in vitro (26). Participation of Cdc42 and PIP2 in activating WASp/Scar does not restrict the upstream pathways, because seven-helix receptors, tyrosine kinase receptors, and integrins can all influence these signaling molecules (59, 60).

WASp-interacting proteins, Grb2, Src kinases, Tec kinases, calmodulin, and Nck are other potential regulators of WASp/Scar proteins (Table I). Like Cdc42 and PIP2, multiple receptor classes might regulate most if not all of these proteins. These proteins could act in the same way as Cdc42 and PIP2, enabling WASp and N-WASP to activate Arp2/3 complex, but they might have other roles such as targeting WASp and N-WASP to particular parts of a cell. Any effect of WASp phosphorylation by Src or Tec kinases is a precedent for other protein activators. Posttranslational modifications of Arp2/3 complex subunits also need to be considered along with differential expression of two p41 ARC isoforms in mammals (10, 61).

Where Does Nucleation Take Place?—It is not clear how WASp/Scar proteins and Arp2/3 complex interact with activators at the leading edge. Because the signal-activating nucleation often comes from a surface receptor and because many of the signal-transducing molecules that bind and/or activate WASp/Scar proteins are bound to receptors or membranes, they may activate Arp2/3 com-

![Fig. 3. Sequence alignment of WASp/Scar activation regions. All are human sequences except Bee1 and Verprolin (budding yeast) and D. Scar (Dictyostelium). Numbers in parentheses represent the starting residue in the alignment. Acidic residues in A region are in red. Highly conserved residues in WH2 motif are in blue.](image)

![Fig. 4. Dendritic nucleation by Arp2/3 complex and WASp/Scar. A, time course of actin polymerization. Final concentrations are: 4 μM Acanthamoeba actin monomers, 15 μM Acanthamoeba Arp2/3 complex, 58 μM Scar1 PWA, 300 μM Acanthamoeba actin filaments. Curves of monomers + Arp2/3 complex or monomers + Scar1 PWA were identical to monomers alone over this time period. This figure was adapted with permission from Ref. 25. B, dendritic nucleation model. Arp2/3 complex binds to the side of a pre-existing filament (yellow), and Scar/WASp (red) bound to an actin monomer binds to Arp2/3 complex in an undetermined order, forming a nucleus for barbed end growth from the side of the filament.](image)
plex on the membrane (26). However, Arp2/3 complex is concentrated in the cortical actin filament network rather than on membranes (9, 10, 12, 13, 61, 62). Additional work is required to pinpoint the sites of Arp2/3 complex activation.

**What Is the Role of Actin Monomer Binding by WASp/Scar Proteins?**—The C terminus of WASp/Scar binds monomeric actin (24, 30, 42), which might stabilize the nascent filament during nucleation (Fig. 4B). However, the way in which WASp/Scar C termini bind actin is not clear. In our hands, human WASp WA binds monomeric actin with a $K_d$ of about $0.5 \mu M$. It inhibits elongation at the pointed end but does not inhibit barbed end growth or sequester actin monomers. Scar1 behaves similarly (24). Others find that N-WASP WA and Scar1 WA polymerize actin filaments (30, 40, 42, 45). One hypothesis proposed for N-WASP WA is that it severs actin filaments, thereby exposing new barbed ends for rapid elongation (40). However, the data for severing could be interpreted in other ways, such as actin monomer sequestration.

In our hands, neither WASp WA nor Scar1 WA sequesters filaments or sequesters monomers.2

**Does Profilin Participate in Actin Nucleation by Arp2/3 Complex and WASp/Scar Proteins?**—Profilin is thought to participate in actin dynamics as a nucleotide exchange factor (63) and carrier for subunits during elongation (64), but the role of its interaction with proline-rich sequences of WASp/Scar proteins in nucleation by Arp2/3 complex is unclear. WASp/Scar family members contain at least four potential profilin-binding sites of 5 or more prolines (Fig. 2), and some evidence suggests that profilin enhances the cellular activities of N-WASP and Scar1 (42, 56). In vitro profilin reduces background nucleation, increasing the signal to noise of the Arp2/3 complex/Scar trigger for new filament formation (25). Scar1 PWA (with potential profilin-binding sites) is more effective than Scar1 WA in the presence of profilin, but the rate is lower than without profilin. Thus profilin interaction with the P domain does not enhance the activation of Arp2/3 complex. One idea is that profilin bound to polyproline-containing ligands targets actin to nucleation or elongation sites (65). We question this theory, because participation of profilin could only slow down diffusion-limited actin filament elongation rate and profilin inhibits nucleation.

**Final Comments**

The recent results summarized here show that WASp/Scar proteins stimulate the formation of new actin filaments by Arp2/3 complex. These fascinating WASp/Scar proteins may in turn be regulated by several signaling pathways. The enthusiasm for these new insights should not lessen the attention given to other complementary mechanisms of actin filament generation such as sequestration. Work also needs to continue on the mechanisms by which actin networks are disassembled to recycle subunits to sites of growth.

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