Published in final edited form as:

Sci Signal. ; 2(55): pe5. doi:10.1126/scisignal.255pe5.

Grab Your Partner with Both Hands: Cytoskeletal Remodeling by Arp2/3 Signaling

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Abstract

The seemingly simple structure of the actin filament belies the elaborate signaling pathways that regulate its assembly and disassembly in eukaryotic cells. In retrospect, this signaling complexity should not be surprising. Actin regulates many dynamic cellular processes, including protein and organelle trafficking, establishment of cell polarity, directional migration, cellular traction, and the efficiency of endocytosis. Signaling events that coordinately control actin turnover during these processes must display a high degree of sophistication. Emerging data on an important regulator of actin dynamics, the actin-related protein 2 and 3 (Arp2/3) complex, suggest a model of how this may occur.

The slow step in filament formation—the association of three actin subunits that initiates or nucleates subsequent de novo polymerization—is key to the cellular assembly of actin. Because this association does not occur efficiently in cells, it represents an important regulatory mechanism to modulate actin dynamics. Several cellular factors enhance this process, such as formins, spire, cordon-bleu, and the actin-related protein 2 and 3 (Arp2/3) complex (1–3). The best characterized of these is the Arp2/3 complex, which consists of two actin-related subunits (Arp2 and Arp3) that mimic actin [for a review, see (4)]. Arp2/3 binds to the side of existing actin filaments, aligning in a similar manner as an actin dimer so that the addition of a single actin monomer potently stimulates actin polymerization (5,6). Consequently, actin structures that arise from Arp2/3 are branched, appearing as a “Y” within dense webs of actin. Arp2/3 promotes the assembly of actin networks very poorly on its own and requires activation by nucleation-promoting factors (NPFs) for optimal activity (3,7,8).

Members of the Wiskott-Aldrich syndrome protein (WASP) family are multidomain NPFs that organize signaling complexes to activate Arp2/3 at the proper time and in the correct spatial location (9). This family contains two categories of proteins: (i) WASP and neuronal WASP (N-WASP), and (ii) Wiskott-Aldrich verprolin homologous proteins (WAVE) 1, 2, and 3. Members of the WASP and WAVE family contain a conserved verprolin-cofilin-acidic region (VCA) domain at the C terminus, which binds to and activates Arp2/3 (10). The ability of these proteins to activate Arp2/3 is tightly controlled by the combinatorial effects of the signaling complexes they organize. The prevailing view is that the VCA domain of the WASP and WAVE family is masked either by intramolecular interactions (in the case of WASP and N-WASP) or by an associated protein complex (in the case of the WAVE proteins). These inhibitory interactions are relieved by allosteric mechanisms that release the VCA domain and that require the activity of Rho GTPases (11–14). Accumulating evidence, however, suggests that a simple switch from inactive to active forms of WASP family proteins is not the complete story of how their activity is regulated. Numerous studies of the WASP and WAVE family have shown that phosphoinositide binding, Src homology 3 (SH3) domain binding, and clustering of WASP or WAVE family proteins favor activation without an apparent allosteric

effect (12,15–21). Additionally, dimeric VCA domains activate Arp2/3 more potently than monomeric VCA domains (15). Furthermore, WAVE proteins can form homo- and heterodimers in vivo, suggesting that dimerization does occur, which may add an additional level of complexity to the signaling complexes they organize (22). How might clustering, SH3 domain binding, or dimerization regulate the activity of WASP and WAVE family proteins? Padrick *et al.* have now analyzed the role of dimerization in regulating the WASP family (23). Their work shows that dimerization can explain how different components of the WASP and WAVE family signaling complexes enhance the activation of Arp2/3.

Using several independent methods, Padrick *et al.* first showed that dimerization of WASP VCA domains was truly a potent mechanism to activate Arp2/3 (23). This heightened activity was attributed to a substantial increase (100 to 180 fold) in the affinity of the WASP VCA dimer for Arp2/3, which resulted in the interaction of two VCA domains per Arp2/3 complex. Did these results reflect the normal mechanisms that govern the activity of WASP proteins? To answer this, the authors tested the effect of PACSIN2 (protein kinase C and casein kinase substrate in neurons 2) on WASP activity. PACSIN2, which binds to WASP through a C-terminal SH3 domain, dimerizes by an N-terminal F-BAR [Fes-Cdc42-interacting protein 4 (CIP4) homology (FCH) and bin-amphiphysin-Rvs] domain. In addition to mediating dimerization, F-BAR domains bind to phosphoinositide-containing membranes and cause the formation of tubular invaginations (24,25). Several F-BAR domain-containing proteins bind members of the WASP and WAVE family and have emerged as important adaptors that link membrane dynamics to the actin cytoskeleton. To exclude potential effects of PACSIN2 on allostery, a WASP mutant lacking autoinhibitory, and therefore allosteric, regulation (WASPc*) was used in actin polymerization assays. When PAC-SIN2 was added to purified WASPc* and Arp2/3, a twofold increase in activation was observed. The authors referred to this elevated activity as hyperactivation. This effect was reversed by adding monomeric PACSIN2 SH3 domain, showing that hyperactivation depends on WASPc* oligomerization by PACSIN2 dimers. Collectively, these data suggested that F-BAR-containing ligands of the WASP family may enhance their activity by inducing dimerization.

Interestingly, many F-BAR- and SH3-containing adaptor proteins [including TOCA-1 (transducer of Cdc42-dependent actin assembly 1), CIP4, and FBP17 (formin-binding protein 17)] that bind the WASP family can also bind dynamin through the SH3 domain (24). The coordinated binding of dynamin, a GTPase involved in endocytosis, and WASP proteins by these adaptors may be one mechanism linking WASP to clathrin-mediated endocytosis. An intriguing issue raised by Padrick *et al.* (23) is whether a predicted competition between WASP and dynamin for interaction with the same F-BAR and SH3 domain-containing adaptor proteins affects the regulation of WASP family hyperactivity at sites of endocytosis.

Binding of phosphatidylinositol biphosphate (PIP₂) can also lead to hyperactivation of WASP. PIP₂, which binds directly to a region of N-WASP that is rich in basic residues, synergizes with the Rho family GTPase Cdc42 to activate N-WASP (15,17,19). Padrick *et al.* speculated that the close proximity of N-WASP on PIP₂-dense vesicles might mimic the effect of VCA domain dimerization on the ability of N-WASP to activate Arp2/3 (23). Indeed, PIP₂-dense vesicles hyperactivated N-WASP, and the degree of hyperactivation correlated with the density of PIP₂ in the vesicles, suggesting that the physical grouping of N-WASP on these vesicles was required. These results suggest that mechanisms that cluster WASP family members will increase their potency toward Arp2/3.

The results of Padrick *et al.* suggest that VCA domain-containing activators of Arp2/3 dimerize to achieve full potency (23). How these results extend to the WAVE subfamily is not yet clear, but it seems likely that these proteins will also be activated by dimerization. For example, the insulin receptor tyrosine kinase substrate p53 (IR-Sp53) contains an inverse-BAR (I-BAR)

domain that dimerizes and an SH3 domain that binds to WAVE2 (26–28). Thus, it is possible that some of the stimulatory effects of IRSp53 may be mediated by dimerization of WAVE2. Likewise, WAVE proteins bind phosphoinositides, and therefore membrane clustering may also regulate their activity (29). Because Arp2/3 contains two binding sites for VCA-containing proteins, the study of Padrick *et al.* (23) also has implications for newly discovered Arp2/3 activators such as WASH [WASP and Scar (suppressor of cyclic AMP receptor) homolog] and WHAMM (WASP homology associated with actin, membrane, and microtubules) that use VCA-like sequences (30,31). Currently, very little is known about the mechanisms that modulate their activity. In light of these studies, it seems likely that signaling complexes to regulate their dimerization may also increase their potency in vivo.

To date, most studies have focused on signaling mechanisms that activate Arp2/3. The notion that VCA dimerization is an important feature of Arp2/3 activation, however, may have important implications for its inactivation. This idea is best highlighted by PICK1 (perinuclear binding protein and substrate for protein kinase C), a neuronal BAR domain-containing protein that regulates postsynaptic trafficking of glutamate receptors. Rocca *et al.* identified a sequence in PICK1 that bears similarities to VCA domains and suspected that it might regulate Arp2/3 (32). Surprisingly, PICK1 inhibited VCA domain-stimulated activation of Arp2/3. Further analysis showed that PICK1 displaced dimeric VCA domains from Arp2/3. PICK1 also directly inhibited the nucleating activity of Arp2/3, suggesting that its inhibitory effects were not only a result of competition with VCA domains. Because PICK1 is a dimer (BAR domains dimerize in a similar manner to the related F-BAR and I-BAR domains), it may effectively compete for both VCA-binding sites on Arp2/3. In light of the studies of Padrick *et al.* (23), PICK1 should strongly oppose Arp2/3 activation or hyperactivation in vivo. Consistent with this prediction, the ability of PICK1 to inhibit Arp2/3 was critical for its regulation of neuronal morphology and trafficking of AMPA-type glutamate receptors at neuronal synapses (32).

Because actin structures undergo rapid remodeling in vivo, mechanisms that de-branch actin filaments and recycle the activity of NPFs and Arp2/3 must exist. But how might these processes be achieved? The finding that VCA dimerization regulates Arp2/3 activation may be salient here as well. For example, cortactin binds Arp2/3 through its N-terminal acidic region (NtA), which shares homology with VCA domains (33). The interaction of cortactin with Arp2/3 stabilizes Arp2/3-containing actin branches, but also displaces the VCA domain of WASP from Arp2/3 (33,34). This may play a vital role in recycling WASP or WAVE proteins so that they can nucleate new filaments, analogous to how actin monomers from depolymerized filaments are reused. Padrick *et al.* showed that dimerized VCA domains were more effective than monomeric VCA domains in competing with cortactin for Arp2/3 (23). Thus, regulation of VCA domain dimerization may alter the ability of cortactin to effectively compete for Arp2/3 in vivo. Remarkably, a Coronin 1B and Slingshot phosphatase complex can also antagonize both cortactin and Arp2/3. Using a combination of single-molecule imaging and cellular assays, Cai *et al.* convincingly show that the Coronin 1B and Slingshot complex replaces Arp2/3 within actin branches and ultimately stimulates actin debranching (35). How Arp2/3 is marked for removal by the Coronin 1B and Slingshot complex is unknown. An appealing possibility is that ATP hydrolysis by Arp2 or Arp3 (36) could act as the timing mechanism to identify “old” Arp2/3 that requires replacement. Nonetheless, in conjunction with the cortactin-mediated release of WASP family members from actin branch points, Coronin 1B complexes may help to reclaim Arp2/3 for reiterative cycles of actin nucleation and branched filament assembly.

From these studies, a speculative, yet holistic, model emerges that may further clarify Arp2/3-mediated actin reorganization (Fig. 1). Signaling ligands act in concert to drive allosteric relief of inhibition of WASP and WAVE family members as well as VCA domain dimerization, which together stimulate actin assembly. Cortactin and Arp2/3 binding releases WASP or WAVE protein from the actin branch, and finally, Coronin 1B and Slingshot recruitment

initiates the disassembly of branched filaments. The fascinating details of how these steps are orchestrated within the cell will surely yield new twists to this evolving story.

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37. I thank members of the Soderling Laboratory and my colleague Terry Lechler for critical reading and comments for this perspective. This work was supported by NIH grant NS059957 to S.H.S.

Fig. 1.

Model of actin remodeling through Arp2/3 signaling. (A) Wiskott-Aldrich syndrome protein (WASP) and Wiskott-Aldrich verprolin homologous protein (WAVE) family members are held in an inactive conformation, which occludes the verprolin-cofilin-acidic region (VCA) domain. The actin-related protein 2 and 3 (Arp2/3) complex is also inactive and may be held in an inhibited state by other proteins, such as perinuclear binding protein and substrate for protein kinase C (PICK1). (B) WASP proteins are activated downstream of Cdc42. This causes an allosteric change that allows the VCA domain to bind and activate Arp2/3. Dimerization of WASP by proteins containing F-BAR [Fes-Cdc42-interacting protein 4 (CIP4) homology (FCH) bin-amphiphysin-Rvs] and SH3 (Src homology 3) domains causes hyperactivation of Arp2/3 by allowing two VCA domains to interact with each Arp2/3 complex. [Clustering of WASP by phosphatidylinositol biphosphate (PIP₂) brings VCA domains in close proximity and also leads to hyperactivation of Arp2/3.] Together, these inputs stimulate the polymerization of a new actin filament along the side of an existing filament at a fixed 70° angle. (C) Cortactin binds Arp2/3 through the N-terminal acidic (NtA) region and can stabilize the actin branchpoint as well as displace WASP and WAVE family proteins. (D) Coronin 1B and the phosphatase Slingshot replace Arp2/3 from the branchpoint. Coronin 1B-containing branches are positioned at variable angles from the existing actin filament compared with Arp2/3-containing branches (indicated by the double arrow). (E) Branches containing Coronin 1B and Slingshot are unstable and lead to the disassembly of the branched actin filament.