Point of VIEW

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Decoupling the Coupling: Surface Attachment in Actin-Based Motility

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ABSTRACT Actin filament polymerization provides the driving force for several kinds of actin-based motility, propelling loads such as the plasma membrane at the leading edge of a crawling cell, an endosomal vesicle, or an intracellular bacterial pathogen. In these systems, branched filament networks continuously grow while simultaneously remaining attached to the load. Previous experiments have suggested an important role in both actin filament nucleation and filament attachment for a family of proteins called nucleation-promoting factors (NPFs) that stimulate actin branch formation and nucleation by the Arp2/3 complex. A recent report demonstrates that N-WASP, an NPF, uses distinct domains to mediate nucleation and attachment during motility. The surprising details of the biochemical mechanism necessitate reconsideration of the biophysical models proposed for actin-based motility.

Some of the greatest difficulties in uncovering the internal workings of biological systems by way of experimental biochemistry come from the intricate and context-dependent behavior of the molecules involved. Studying pairwise interactions between the relevant molecules may not reveal all the information needed to explain the behavior in context, where one interaction may be modulated or precluded by another, or even become indistinguishable from a parallel interaction mediated by a separate set of molecules. Moreover, molecules assemble into dynamic structures with complex architectures, causing new behaviors to emerge in the system as a whole.

Actin-based motility is one system in which such complex molecular assemblies have continued to perplex scientists trying to elucidate the underlying molecular mechanisms. In organismal development, wound healing, cancer metastasis, and other processes, migrating cells extend projections known as lamellipodia or filopodia, which contain branched networks or cross-linked bundles of actin, respectively. In the case of the lamellipodium, filament branching is mediated by the Arp2/3 complex (*1*), which, when activated, binds to the side of an existing ("mother") filament and nucleates the polymerization of a new ("daughter") filament (*2*). Continuous extension of the lamellipodium is thought to occur through filament nucleation at the leading edge, balanced by depolymerization at a posterior zone (*3*), although measurable actin filament assembly and disassembly occur throughout the entire structure (*4*). Enhancement of nucleation at the leading edge is achieved through specific nucleation-promoting factors (NPFs), such as proteins of the WASP/WAVE family, which are capable of activating Arp2/3 and reside on the leading-edge membrane (*5*) (Figure 1).

The biochemical dissection of the actin-based protrusion machinery has been greatly facilitated by the existence of motile intracellular pathogens that highjack the host-cell actin system to propel themselves, by mechanisms that share important components with the leading-edge machinery (*6*). *Shigella flexneri*, a Gram-negative bacterium that causes epidemic dysentery, recruits the NPF N-WASP to its surface, causing the formation of an actin "comet tail" that pushes it through the host-cell cytoplasm and facilitates its transmission to neighboring cells. The Gram-positive *Listeria monocytogenes*, which causes a rare but serious form of food poisoning, uses its own protein, ActA (Figure 1), to activate the Arp2/3 complex in a roughly analogous manner. Bacterial motility can be reconstituted in an *in vitro* system where the cytosol is replaced by a defined mixture of purified components (*7*). Furthermore, artificial objects such as plastic beads (*8*) or lipid vesicles (*9, 10*) coated with the bacterial protein ActA or with another NPF such as N-WASP (*11*) are also able to form actin comet tails and move in a remarkably lifelike manner in cytoplasmic extracts or reconstituted systems.

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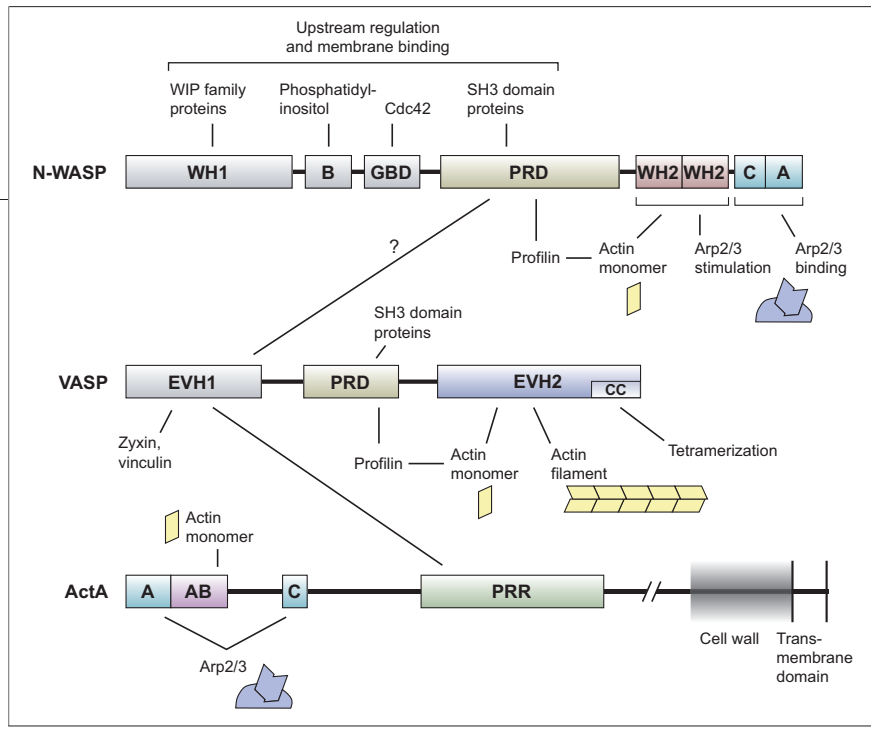

Figure 1. Multidomain proteins controlling polymerization in actin-based motility. N-WASP (top) is a WASP-family protein (*5*). The N-terminal portion carries domains that interact with factors responsible for regulation and localization (WH1, WASP-homology 1; B, basic segment; GBD, GTPase binding domain; PRD, proline-rich domain). The proline-rich domain also binds profilin, an actin monomer binding protein. The WH2 (WASP-homology 2) domains bind actin monomers and enhance Arp2/3 stimulation. The C (central or cofilin-homology) and A (acidic) regions bind the Arp2/3 complex. VASP (middle) is a member of the Ena/VASP family (*22*). The EVH1 (Ena/VASP-homology 1) domain has sequence similarity with WH1 domains but binds a different set of factors, including ActA and possibly WASP, a paralog of N-WASP. The proline-rich domain, as in N-WASP, binds profilin and other factors. The EVH2 (Ena/VASP-homology 2) domain binds actin monomers and filaments at distinct subdomains and includes a coiled-coil segment involved in homotetramerization. ActA (bottom) is the *L. monocytogenes* surface motility factor. The N-terminal domain contains acidic (A) and cofilin-homology (C) segments with sequence similarity to the corresponding domains in WASPs, as well as an actin monomer binding region (AB) (*23*). The proline-rich repeat (PRR) domain differs from the proline-rich domains of WASPs and VASP and recruits Ena/VASP proteins *via* their EVH1 domain (*22*).

The growth of an actin network at the surface of an intracellular bacterium or at the leading edge of a cell is coordinated over the spatial scale of thousands of molecules and can persist for a time scale of hours. For an actin network to grow in such a manner, the growth of filaments must be strictly limited to the bacterial surface or to specific and consistent regions of the plasma membrane, coinciding with localized NPF activity. One conceivable way in which the cell might accomplish this is through a mechanical link between actin filaments and the leading-edge membrane (or bacterial surface) that can keep the growing barbed ends positioned close to the NPFs, thereby allowing continuous and coordinated network growth.

Such an attachment has indeed been shown to exist. Kuo and McGrath (*12*) showed that the diffusion coefficient of *L. monocytogenes* undergoing intracellular motility is much smaller than expected for a freely diffusing object of the same size. Gerbal *et al.* (*13*) used optical trapping to directly demonstrate the firm attachment of the actin tail to an ActA-coated plastic bead. Marcy *et al.* (*14*) found that pulling gently on the load object with a micromanipulator did not induce detachment and, intriguingly, slightly accelerated actin network growth. Using electron microscopy, Cameron *et al.* (*15*) found individual filaments that appeared to be attached to the motile beads.

These observations raise a puzzling conceptual question: how can the network of actin filaments remain attached to the load while simultaneously polymerizing and pushing on the load? Two broad categories of physical models have been proposed. First, because force-generating actin networks typically contain hundreds or thousands of filaments, it is possible that some of the filaments in the network might be attached to the surface (perhaps *via* a ternary binding complex consisting of the surface-attached NPF, the Arp2/3 complex, and the actin filament; Figure 2, panel a) while other filaments in the network are free to polymerize and push (*16*). Alternatively, specialized filament end-tracking proteins may be able to maintain a binding interaction with the tip of a growing filament while still permitting polymerization (*17*). The molecular identity of the filament–surface attachment, however, remained poorly understood, with limited and indirect experimental evidence supporting each model of attachment. A particularly tricky complication has been the fact that the NPFs, including N-WASP and ActA, as well as several of their binding partners such as Ena/VASP, are highly complex multidomain proteins that harbor multiple distinct binding sites for filamentous actin, monomeric actin, various other actin binding proteins, and one another (Figure 1). A recent report by Co, Taunton, and coworkers (*18*) uses a systematic mutagenesis strategy with a well-designed *in vitro* assay to provide fresh insight into this problem.

Co *et al.* (*18*) used an *in vitro* system consisting of lipid vesicles, purified N-WASP, and *Xenopus* egg cytoplasmic extract, in which N-WASP binds to the vesicle surface and causes the vesicle to undergo comet-tail motility. Because N-WASP, able to diffuse freely on the vesicle surface, was localized asymmetrically to the region of the vesicle where the tail was attached, different fluorescently labeled fragments of N-WASP were introduced into the system to see whether they would also localize to the same zone, as would be expected for a fragment capable of binding to actin filaments in the tail. Interestingly, the authors observed that fragments lacking the CA domain, which is known to bind Arp2/3, were able to localize asymmetrically, whereas those lacking the WH2 domains, known to

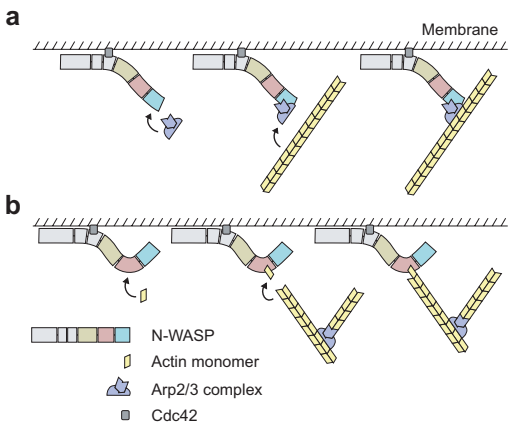

Figure 2. Models for actin network attachment *via* membrane-tethered N-WASP. N-WASP is tethered to the membrane through its activator, Cdc42 (cell division cycle 42), and through phosphatidylinositol binding *via* its basic domain. a) The Arp2/3-mediated attachment model. If N-WASP-bound Arp2/3 could simultaneously bind to an existing filament, then the ternary complex could mediate attachment. No direct evidence exists that such a ternary complex is formed, and Co *et al.* (*18*) demonstrate that this mode of attachment is neither necessary nor sufficient for gross actin network attachment. b) The filament barbed-end attachment model, suggested by Co *et al.* (*18*). A WH2 domain on N-WASP binds to an actin monomer, which can capture a filament barbed end by monomer addition. Whether the Arp2/3 stimulation activity of N-WASP is coupled to this interaction remains unknown.

bind actin monomers, were uniformly distributed on the vesicle surface, an indication of an attachment defect. In fact, when one of the two WH2 domains was deleted, two single-residue mutations in the other were each sufficient to cause the loss of asymmetric localization.

Both point mutations were also associated with decreased binding affinity for actin monomers. Yet, the mutations did not detectably alter N-WASP's activity to stimulate actin nucleation *via* Arp2/3. This made it possible for Co and coworkers to elegantly separate the tail-construction and tail-attachment activities of N-WASP in a reconstituted system consisting of purified proteins and lipid-bilayer-coated glass beads. In the assay, the lipid-coated beads formed actin comet tails and moved in the precence of activated N-WASP, Arp2/3 complex, actin, capping protein, and profilin. When the N-WASP was substituted with one of the two mutants, however, the beads would dissociate from the tails at near-100% frequency in <1 h, even though the tails appeared normal. This suggested a correlation of tail attachment to actin monomer binding, rather than to Arp2/3 binding. In fact, when Arp2/3 was competitively inhibited during motility, the beads remained attached to the tails even after halting.

Co and colleagues (*18*) further investigated the requirement for the N-WASP WH2 domain to bind actin filaments. N-WASP-coated beads could capture fluorescently labeled actin filaments if, and only if, actin monomers were present and the N-WASP bore an intact WH2 domain. The capture was antagonized by capping protein, an indication that WH2 was binding to the barbed end of the filaments. By means of a carefully designed N-WASP replacement experiment, the authors showed that a WH2 domain is not only necessary but also sufficient to mediate attachment to the tail: an N-WASP fragment with only the WH2 and CA domains could replace wild-type N-WASP without loss of attachment, whereas the CA domain alone was insufficient. Thus, N-WASP-mediated attachment of a membrane to a growing actin network does not have to be bridged by Arp2/3 (as in Figure 2, panel a) but occurs through the capture of filament barbed ends, possibly bridged by a prebound actin monomer (Figure 2, panel b). This specific mechanism had not been previously predicted by any of the physical models for this system.

The success of Co *et al.* (*18*) depended on the ability to isolate the effect of one function of a protein (attachment to filaments) from others (polymerization and motility) without completely dismantling the architecture of molecules that generates large-scale behavior. That enabled them to rule out proposed models that were based on knowledge of pairwise interactions of the components. The known interaction of Arp2/3 with both N-WASP and actin filaments had tempted the speculative extrapolation that Arp2/3 may mediate attachment, although this is not consistent with structural evidence that the NPF does not remain associated after branch formation (*19*).

The dispensability of Arp2/3 for maintaining attachment is particularly interesting in light of an earlier observation by Brieher *et al.* (*20*) that under certain conditions, *L. monocytogenes* could continue to move (and remain attached to a structurally altered comet tail) after Arp2/3 was inhibited. Because it is also known that VASP, another protein recruited by ActA, is not essential for motility (or attachment) (*21*), ActA's own capacity to bind actin filaments may be responsible for tail attachment in this case. Curiously, though, Co *et al.* (*18*) observed a slight increase in speed when barbed-end attachment was compromised, whereas Brieher *et al.* (*20*) found that speed was greatly increased and dependent on actin concentration when Arp2/3 was inhibited. This may be due to differences between N-WASP- and ActA-mediated motility and leads to the question: how general is the mechanism of attachment described by Co and coworkers?

Slight differences exist among WASP/WAVE-family proteins in domain organization and function, and they appear to be involved in different processes within the cell. WAVEs are essential at the leading edge, whereas WASP and N-WASP seem to be involved in endocytosis and vesicle motility (*5*). In addition, proteins of the Ena/VASP family, which share some similar domains with WASPs (but with slightly different properties) (Figure 1), also reside at the leading

edge as well as the surface of *L. monocytogenes*, are capable of binding monomeric and filamentous actin, colocalize with WASP/WAVE proteins, and bind to WASP (*22*). A full understanding of filament–surface interaction will require further investigation of the role played by these and other proteins, including possible alternative or redundant mechanisms for attachment.

In the particular case of N-WASP-mediated motility, the architecture of the tail created behind beads appears to be highly regular. Is this a result of some precise balance that N-WASP maintains between filament elongation and branching/nucleation, or is it simply a result of individual rate constants? Binding of barbed ends is likely to have some effect on filament elongation, but what that effect is and whether there is communication between the barbed-end binding activity and the Arp2/3 stimulating activity remain interesting mechanistic questions.

In the last decade or so, tremendous progress has been made in the biochemical reconstitution of spatially organized processes at an increasingly complex scale, without giving up the exquisite control afforded by the use of well-defined components. Actin-based motility, as described here, is just one of the many important cell biological processes, including signal transduction, chromosome segregation, cell adhesion, and tissue formation, that are currently being dissected in this way. Decoupling subprocesses within such complex systems will continue to be an important challenge for biochemists and, in many cases, is likely to alter our conceptual understanding of the fundamental mechanisms of their behavior and function.

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