Progress in Protrusion: The Tell-Tale Scar

Tatyana M. Svitkina and Gary G. Borisy

Laboratory of Molecular Biology, University of Wisconsin, Madison, Wisconsin 53706, USA

Address correspondence to:
Gary G. Borisy
Laboratory of Molecular Biology
Bock Laboratories
University of Wisconsin
1525 Linden Drive
Madison, WI 53706
Tel: (608) 262-1365
Fax: (608) 262-4570
email: g-borisy@northwestern.edu

Abstract

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ABSTRACT ↑

The crawling movement of a cell involves protrusion of its leading edge coordinated with translocation of its cell body and depends upon a cytoplasmic machinery able to respond to signals from the environment. Protrusion is now understood to be driven by actin polymerization and signalling from membrane receptors to actin has been shown to be mediated by the Rho family of GTPases. However, a major gap in our understanding of regulated motility has been to connect the signalling pathway to the motile machinery itself. Recent structural, biochemical and genetic studies have identified some of the missing links and provided a strong working model for the pathways and mechanism by which the signals are interpreted and implemented.

Two major protrusive organelles exist in crawling cells: lamellipodia containing a network of diagonally oriented actin filaments and filopodia containing a tight bundle of parallel actin filaments, the filaments invariably oriented with their barbed ends forward. Models proposed to account for polymerization-driven protrusion of lamellipodia have involved actin filaments being nucleated at the membrane and subsequently released[1] or continuously treadmilling by growth at their barbed end and shortening at their pointed end[2].

Recent electron microscopic studies[3,4] have revealed new details of the supramolecular organization of actin filaments in lamellipodia. The leading edge is characterized by a distinctive, extensively branched network of actin filaments. Consistent with previous models for protrusion, barbed ends were numerous near the leading edge but, surprisingly, free pointed ends were not detectable. Instead, pointed ends were involved in structural association with the sides of other filaments at ~70 degree angles resulting in Y-junctions (Fig. 1A) and formation of a diagonal network filling the lamellipodium. These structural studies suggested that lamellipodial protrusion needed to be re-interpreted in terms of the formation and recycling of a branched filament network

Arp2/3 complex ↑

A key component in understanding the origin of the branched network is the Arp2/3 complex (reviewed in Refs.5 and 6). It consists of actin-related proteins 2 and 3 and five other proteins[7-10], it localizes to the leading edge of crawling cells[7-9,11], to cortical actin patches in yeast[10], and it is sufficient to induce actin

polymerization at the surface of Listeria cells[12]. The Arp2/3 complex binds to the sides of actin filaments and to their pointed ends[8] and nucleates actin filaments creating ~70 degree branches on pre-existing filaments[13] (Fig. 1B) similar to those seen *in vivo*[3]. These structural, functional and biochemical data strongly suggested a dendritic nucleation model[13] for the mechanism of actin polymerization in cell protrusions in which formation of new actin filaments occurs by Arp2/3-mediated nucleation on pre-existing filaments, thus resulting in assembly of a branched network. Further support for this model comes from the recent demonstration that the Arp2/3 complex localizes to Y-junctions at the leading edge in vivo[4] (Fig. 1A, inset). A principal feature of the model is that tightly coupled nucleation and cross linking of actin filaments at the leading edge would allow nascent filaments to push against the membrane immediately after formation and provide the structural basis for polymerization-driven protrusion.

WASP-family proteins: how the Arp2/3 complex is activated ↑

A problem with the dendritic nucleation model was that the intrinsic nucleating activity of the Arp2/3 complex is weak. Thus, some stimulatory mechanism seemed to be required. Evidence pointing to regulation of the Arp2/3 complex originally came from the study of Welch et al.[14] showing that the Listeria protein, ActA, significantly enhances the nucleating activity of the Arp2/3 complex. Subsequently, cellular analogues of ActA have been identified[15] as members of the WASP (Wiscott-Aldrich Syndrome protein) family (reviewed in Ref. 16). The members tested to date, including WASP[17], N-WASP (neuronal -WASP)[18], Scar/WAVE (suppressor of cyclic AMP receptor/WASP-family verprolin-homologous)[19], and Las17p/Bee1p (similar to WASP)[20], all enhanced Arp2/3-mediated nucleation in vitro. WASP and N-WASP are closely related proteins with different expression patterns: WASP is expressed in hematopoietic cells, whereas N-WASP is more ubiquitous. Las17p/Bee1p is a yeast homolog of WASP[21]. Scar/WAVE[22,23] has extensive homology with WASP and N-WASP in its C-terminal domain but has a different N-terminal domain[15,23].

The homologous C-terminal regions of WASP-related proteins play a key role in activation of actin filament nucleation by Arp2/3 complex. The necessary and sufficient domain for stimulation consists of three motifs: verprolin homology (V) also called WASP-homology 2 (W or WH2), cofilin-homology (C), and acidic (A)[18-20]. The V-motif binds G-actin[15,24], whereas the CA motifs together bind the p21 subunit of the Arp2/3 complex[15]. In yeast, two subunits of the Arp2/3 complex, Arc15p and Arc19p, are involved in interaction with the WASP homologue, Las17p/Bee1p20. Stimulation of nucleation may result from the VCA domain binding both the Arp2/3 complex and an actin monomer and bringing them together in appropriate orientation to promote binding, thus forming a seed for further elongation[15]. Based on structural evidence, a detailed model for the putative conformational change in the Arp2/3 complex has been proposed[6].

Although all studied WASP-related proteins caused acceleration of actin polymerization in vitro, they did not eliminate the initial lag phase seen in the conventional biochemical assay. In contrast, preincubation of Scar/WAVE and Arp2/3 complex with actin filaments resulted in elimination of the lag phase[19], strongly indicating that the weak intrinsic nucleating activity of the Arp2/3 complex is activated by interaction with Scar/WAVE and the sides of actin filaments. In the framework of the dendritic nucleation model, these data imply that a branched actin network in lamellipodia is formed by Scar/WAVE-Arp2/3-mediated nucleation off sides of pre-existing actin filaments as opposed to nucleation elsewhere followed by docking of a nascent filament onto the side of another filament. Thus, the discovery of Scar provides the missing link in understanding how the Arp2/3 complex promotes the unique branched network organization of actin filaments in lamellipodia.

Signalling to WASP proteins: formation of filopodia vs lamellipodia ↑

The Rho family GTPases, Cdc42 and Rac have been established as signals for formation of filopodia and

lamellipodia, respectively . In extracts, Cdc42 charged with the GTP analog, GTPgS, induces actin polymerization[26-30]. Proteins of the WASP family provide a potential link between these signalling molecules and the actin cytoskeleton (reviewed in ref. 16). Genetic data demonstrate that the N-termini of WASP and N-WASP contain binding sites for small GTPases called GBD (GTPase binding domain) or CRIB (Cdc42/Rac interactive binding) domain, which can directly bind Cdc42 and, more weakly, Rac[31,32]. The N-termini also bind phosphoinositides[33] through what was originally thought to be a PH (pleckstrin homology) domain but which subsequently was shown to be an EVH1 (Ena/VASP homology) domain[34].

The domain organization of proteins of the WASP family suggests a mechanism for their regulation by Cdc42. The highly acidic VCA domain of N-WASP was proposed to be involved in an autoinhibitory intramolecular interaction with the basic region at the N-terminus next to the GBD/CRIB domain[18,35]. Cdc42 binding disrupts this interaction and releases the VCA domain, leading to activation of N-WASP[35]. N-WASP also can be regulated by PI(4,5)P_2 (phosphatidylinositol-3,4-biphosphate) which had additive effects with Cdc42 on activation of N WASP[18].

The downstream effects of the WASP family members appear to be distinctive. N-WASP, but not WASP has been shown to be a Cdc42-dependent effector for filopodia formation[35,36]. WASP may function in hematopoietic cell lineages in formation of surface structures in T-cells[37] and in directional motility in macrophages[38]. Yeast Las17p/Bee1p, in contrast to WASP, does not contain a GBD/CRIB motif[21] and thus might be regulated differently. In yeast, Las17p/Bee1p is important for formation of actin patches.

In the case of Scar/WAVE, connection to the GTPase signalling system seems to be more complicated. The N-terminus of Scar/WAVE is different from WASP and N-WASP, and does not contain domains which might bind small GTPases. Although biochemical evidence indicates that Scar/WAVE does not bind Rac or Cdc42 directly, Rac was immunoprecipitated by Scar/WAVE antibody[23], suggesting that some adaptor molecule(s) may mediate their association. Even though Scar/WAVE, like N-WASP, contains basic and acidic domains, the activity of full length Scar/WAVE in vitro is not suppressed compared to truncated mutants[19]. Thus, regulation by an intramolecular inhibition mechanism is uncertain.

Several sets of data suggest a role for Scar/WAVE in transmitting signals from Rac to induce lamellipodia. Ectopically expressed Scar/WAVE[23] or just its C-terminus[15] induce an increase in cytoplasmic actin filaments. These effects depend both on actin-binding[23] and Arp2/3 binding[15] domains of Scar/WAVE. Expression of constitutively active Rac induces translocation of Scar/WAVE from the cytoplasm to lamellipodia[23]. Dominant-negative constructs of Scar/WAVE lacking the V-motif specifically block induction of lamellipodia by Rac, but not filopodia by Cdc42[23]. The C-terminus of Scar/WAVE containing the VCA domain displaces Arp2/3 complex and prevents lamellipodia formation[15]. Finally, Scar induces actin filament dependent, actin nucleation in a purified system in vitro[19]. Scar/WAVE may also be involved in other signalling pathway such as those working through heptahelical receptors and heterotrimeric G-proteins[22].

Thus, the emerging picture is that Rho-family GTPases signal the formation of dynamic actin structures through different WASP-family proteins, the pathways converging on the Arp2/3 complex. How different WASP-related proteins using the same intermediate, the Arp2/3 complex, induce different responses in cells remains to be determined. Recruitment of different accessory proteins is likely to be be involved with differential stimulation of nucleating and side-binding activities of the Arp2/3 complex. A recent study on structure and protein composition of comet tails assembled by different intracellular bacteria[39] suggested that Rickettsia exploit a cellular mechanism similar to that for filopodia formation, whereas Listeria and Shigella use a lamellipodial mechanism. Investigation of these model systems opens a possibility to unravel differences in mechanisms of formation of filopodia and lamellipodia.

Targeting to the membrane ↑

Previous models for the mechanism of protrusion assumed that actin nucleation occurred at the plasma membrane, which immediately provided a mechanism for targeting actin polymerization to the leading edge. However, in the dendritic nucleation model, nucleation occurs not at the membrane but on pre-existing filaments which raises the problem of the relationship between the actin network and the membrane.

Ectopic expression of WASP-related proteins frequently results in aberrant actin polymerization[23,15,32], probably because of failure of the expressed protein to localize properly. To test this possibility, Castellano et al.[40] developed a system for controlled clustering of constitutively active Cdc42 or WASP at the cytoplasmic face of the plasma membrane. This approach was based on the ability of a small permeable molecule, rapamycin, to bind two different ligands. One of these ligands was fused with constitutively active Cdc42 and another with the cytoplasmic domain of a surface receptor, which can be clustered by antibody-coated beads. Association of Cdc42 with the membrane in normal conditions occurs via its C-terminal membrane-binding domain[28]. When both beads and rapamycin were added, clustering of Cdc42 at the bead-membrane interface resulted in filopodia protrusions with beads associated with filopodial tips. When WASP was used instead of Cdc42, filopodia were also formed, but with altered morphology. These data indicate that Cdc42 and/or WASP may recruit Arp2/3 complex to the membrane and thus transform random actin polymerization into functional structures. The Arp2/3 complex was not detected in the induced filopodia, suggesting it initiated the reaction and remained at the base of the induced filopodia or became dissociated from it.

Filopodia induced by controlled clustering of Cdc42 at the membrane[40] recruited vasodilator-stimulated phosphoprotein (VASP) (reviewed in Ref. 41). This is particularly interesting because of recent demonstration of a role for VASP in directional motility of Listeria in cytoplasmic extracts[42]. When VASP was depleted from extracts, diffuse actin clouds but no tails were formed around the bacteria. Addition of VASP or other proteins of the VASP family to depleted extracts restored tail formation and bacterial motility. Investigation of molecular interactions of VASP revealed that the N-terminal EVH1 domain of VASP binds bacterial ActA protein stably and the C-terminal EVH2 domain binds actin filaments more loosely. The authors proposed a model in which VASP works as a sliding molecular connector between the bacterial wall and a growing barbed end of an actin filament. This mechanism allows for insertional actin polymerization, but imposes structural constraint on the position and orientation of growing barbed ends. It is likely that VASP plays a similar role in cells, but uses a different component to link barbed ends to the membrane. Zyxin is a candidate, since VASP interacts with zyxin itself and the zyxin-like proline-rich region of ActA (reviewed in Ref. 43). Further, zyxin became enriched at the tips of filopodia induced by Cdc42 recruited to membranes[40].

Protrusion and treadmilling in the steady-state ↑

Similar but distinct mechanisms can be proposed for initiation and steady-state protrusion of filopodia and lamellipodia (Fig. 2). The existing data about filopodia are consistent with a single event of nucleation resulting from interaction between activated N-WASP and the Arp2/3 complex, followed by continuous elongation of barbed ends at the membrane. In the course of elongation, the Arp2/3 complex remains at the pointed end and detaches from membrane-associated proteins. Actin depolymerization in filopodia is likely to occur at pointed ends at the base of a filopodium. This model predicts that no Arp2/3 complex or actin depolymerizing factor(ADF)/cofilin will be associated along the length of the filopodium, consistent with experimental observation[4].

In lamellipodia, the high frequency of actin filament branching near the leading edge[4] suggests a high level of actin filament nucleation. Does this result mean that all branches grow continuously or are new filaments capped soon after nucleation and only a small proportion of them continue to elongate and branch? Several lines of evidence support the latter possibility: (1) The vast majority of barbed ends is capped and stimulation

of actin polymerization in cells leads to association of capping protein with the cytoskeleton[44]; (2) capping protein localizes to the leading edge[11]; (3) actin seeds become capped soon after addition to extracts[27], and (4) uncapped barbed ends, were found in lamellipodia only within 0.1-0.2 μm from the leading edge[45], whereas apparently free barbed ends (capped and uncapped) were abundant within 1 μm from the leading edge[4]. Thus, it seems likely that most actin filaments at the leading edge become capped at their barbed ends shortly after they are formed.

The Arp2/3 complex has been shown to cap pointed ends in vitro[13] and protect branched actin network in lamellipodia from depolymerization in vivo[4], thus hindering depolymerization of actin filaments. Consequently, mechanisms must exist to overcome this inhibition. ADF/cofilin has been shown to play an important role in the depolymerization of actin filaments during actin based motility (reviewed in Ref. 46,47). Two mechanisms of ADF/cofilin action in actin disassembly have been described,--facilitated release of actin subunits from the pointed end and severing[46,48]. The mechanism or combination of mechanisms ADF/cofilin uses in vivo and how the antagonistic activities of ADF/cofilin and Arp2/3 complex are coordinated in cells are important questions for understanding actin turnover in lamellipodia. One possibility, based on functional and structural studies[4], is that ADF/cofilin binds actin filaments within the actin network and waits for the release of the Arp2/3 complex to get a chance to facilitate subunit dissociation from pointed ends. In this framework, depolymerization and, therefore, actin filament turnover is regulated by signals which induce Arp2/3 dissociation from pointed ends. Recent data showing that ADF/cofilin can facilitate pointed end depolymerization even in the presence of Arp2/3 complex[49] raise the possibility that ADF/cofilin by itself may function as a regulatory signal for Arp2/3 complex dissociation.

The dendritic network model suggests a novel concept of actin turnover in lamellipodia in which the actin array as a whole treadmills but not individual actin filaments per se (Fig. 3). In the array treadmilling model, an individual filament is 'born' at an Arp2/3 branchpoint, grows at the barbed end, becomes capped, and later 'dies' by shortening from the pointed end after dissociation of the Arp2/3 complex. Nevertheless, the array as a whole treadmills, reproducing itself at the cell front and dismantling itself at the lamellipodial rear.

From the functional point of view, a dendritic network of actin filaments at the leading edge of locomoting cells seems well-designed for lamellipodial protrusion. First, the network is mechanically organized for efficient polymerization-driven force generation because of its high filament density, optimal filament length, extensive cross-linking of actin filaments and the angular orientation of actin filaments[50]. Second, the level of polymerization of the network can, in principle, be readily controlled by regulation of the activity of the Arp2/3 complex and/or capping protein. Finally, an array-treadmilling mechanism may play a role in the persistence of lamellipodial protrusion and in the ability to adapt to change in direction. A challenge for future research will be to unravel the precise molecular mechanisms regulating the interplay between structural organization and force generation and the pathways by which signalling molecules determine the mode of organization and function.

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