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Actin Dynamics: Growth from Dendritic Branches

Review

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The dendritic nucleation model was devised to explain the cycle of actin dynamics resulting in actin filament network assembly and disassembly in two contexts — at the leading edge of motile cells and in the actin comet tails of intracellular pathogenic bacteria and viruses. Due to the detailed nature of its biochemical predictions, the model has provided an excellent focus for subsequent experimentation. This review summarizes recent work on actin dynamics in the context of the dendritic nucleation model. One outcome of this research is the possibility that additional proteins, as well as the six proteins included in the original model, might increase the efficiency of dendritic nucleation or modify the resulting actin network. In addition, actin dynamics at the leading edge might be influenced by a second actin filament network, independent of dendritic nucleation.

Actin is one of the most abundant proteins in eukaryotic non-muscle cells, ranging from low micromolar to hundreds of micromolar concentrations. Its roles in cells are highly diverse, and we probably have not yet identified all of them. Many of actin's cellular roles revolve around its 'dynamics', or ability to polymerize and depolymerize rapidly. For this reason,understanding the mechanisms underlying cellular actin dynamics has been a major research focus for many years.

The dendritic nucleation model was proposed in 1998 to explain the formation of branched actin networks nucleated by Arp2/3 complex in motile cells [1]. It was later expanded in 2000 to include explanations of force generation and monomer recycling in these actin networks [2]. This model has had a major effect on the field of actin dynamics, and aspects of the model have been tested in many cellular and biochemical contexts. Our review discusses recent findings in actin dynamics, in the context of the dendritic nucleation model. The first part of the review concerns the roles, or potential roles, of individual proteins in dendritic nucleation. The second part concerns the role of dendritic nucleation in generating actin-based structures in the protrusive region of a migrating cell.

Basic Actin Biochemistry

To understand the dendritic nucleation model, a knowledge of actin dynamics is required [2]. Actin is a 43 kDa monomeric protein that can polymerize into double-helical filaments. The initial stages of polymerization — dimerization and trimerization — are unfavorable and occur slowly (Figure 1A). Addition of subsequent monomers is favorable, and the filament elongates rapidly. Since all subunits (i.e. filament-incorporated monomers) face the same direction, the filament is polar. Monomers add faster to one end (known as the 'barbed' or 'plus' end) than to the other ('pointed' or 'minus' end). The rate constants for these reactions are known [3,4].

Overlaying these aspects of actin polymerization is actin's ATPase activity. Actin monomers bind ATP tightly, and hydrolyze the nucleotide upon addition to the filament. However, under most conditions, hydrolysis occurs with a considerable lag after polymerization. Furthermore, the inorganic phosphate (Pi) product of hydrolysis is released with an even more considerable lag, while the ADP product remains tightly bound. ATP hydrolysis and Pi release affect filament stability, as ADP-bound actin monomers have less affinity for filament ends than do ATP-bound monomers (Figure 1A).

Dendritic Nucleation Model

Only six proteins are required in the model: actin; Arp2/3 complex; an Arp2/3 complex-activating nucleation promoting factor (NPF); a barbed-end capping protein; ADF/cofilin (hereafter called cofilin); and profilin. The details of the model have been described elsewhere [2,5], but we outline the main points here (Figure 1B).

A pool of cytoplasmic actin monomers is bound to profilin, strongly inhibiting spontaneous nucleation. In addition, profilin-bound monomer cannot add to pointed ends, so all monomer addition occurs at barbed ends. Under these conditions, Arp2/3 complex nucleates new filaments by mimicking a pointed end, but must be activated by binding two activators: an NPF and the side of a pre-existing filament. Arp2/3 complex's ability to remain bound to both the pointed end of the new 'daughter' filament and the side of the existing 'mother' filament causes formation of a 70° branch, known as a 'dendritic branch'. This branch formation is one of the central points of the model, and results in a branched network.

After nucleation, monomers (mostly bound to profilin) add to the barbed end and the filament elongates. However, high concentrations of barbed-end capping proteins cause rapid barbed-end capping. Capping serves an essential role in force generation, by maintaining short filaments capable of deforming the membrane to which they abut [6,7].

As filaments in the dendritic network age, their actin subunits hydrolyze bound ATP, and release Pi slowly. Pi release enhances cofilin binding, and cooperative cofilin binding in turn accelerates Pi release. This Pi release acceleration reduces Arp2/3 complex affinity for the pointed end by 20-fold, causing branch

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disassembly. In addition, cooperative cofilin binding to ADP-bound actin subunits causes filament severing. These two factors increase the number of free pointed and barbed ends, allowing depolymerization from these ends.

Many of the depolymerized ADP–actin monomers are bound to cofilin. These monomers release from cofilin and bind profilin, which accelerates nucleotide exchange with abundant cytoplasmic ATP. The profilin-bound ATP–actin monomer can add to barbed ends, completing the cycle and allowing sustained polymerization.

Evidence for the Dendritic Nucleation Model

The groundwork for the model was provided by many years of detailed biochemical studies on the proteins involved (reviewed in [2]), supplying rate and equilibrium constants for most binding and chemical reactions, as well as cellular concentrations for the proteins in several systems. One of the remarkable features of the model is its attention to biochemical and biophysical detail, allowing for rigorous testing. Detailed structural electron microscopy studies have characterized the dendritic branches formed by Arp2/3 complex [1,8].

Studies on actin comet tail formation by the intracellular pathogenic bacterium *Listeria monocytogenes* were also crucial to the model. *Listeria* moves in eukaryotic cytoplasm by initiating formation of an actin-based structure (the comet tail) that pushes it forward [9]. The parallels between this motility system and leading edge motility of motile cells have long been appreciated, and *Listeria* has served as a model for actin-based motility. A breakthrough was the reconstitution of *Listeria* motility with purified proteins [10]. Only five proteins are needed for basal motility: ActA, an NPF for Arp2/3 complex and the sole bacterial protein needed; Arp2/3 complex; capping protein; cofilin; and actin. Profilin addition increases motility approximately threefold, an increase explained in the model by accelerating monomer recycling.

Another crucial result was identification of dendritic networks at the leading edge of motile cells [11], and localization of Arp2/3 complex to the dendritic branchpoints [12,13]. Cofilin and capping protein also localize to this region [12,14–17]. Subsequent electron microscopy has identified dendritic networks in comet tails of *Listeria*, as well as those of *Shigella flexneri* and Vaccinia virus [18,19]. Interestingly, *Rickettsia* bacteria also activate Arp2/3 complex [20,21], but their comet tails contain unbranched filaments [18,22]. Actin patches in budding yeast also contain dendritic branches [23,24]. Another actin-based structure, the podosome, is strongly suspected to be driven by dendritic nucleation, but direct identification of dendritic branches has not been carried out. These basal adhesion structures, found in macrophages and other cells, are dependent on Arp2/3 complex and the NPFs, WASp and N-WASP [25]. Related structures, called invadipodia, have similar requirements and also require cofilin [26].

Additional Proteins that Might Act in Dendritic Nucleation

Information has been gathering on several proteins that might play fundamental roles in cellular actin dynamics. We present these proteins here for two reasons: they are present in all eukaryotes examined; and, where measured, they are present at high cytoplasmic concentrations. These proteins could serve in conjunction with cofilin and profilin to enhance monomer recycling rates.

Srv2/CAP

Srv2/CAP is a 57 kDa monomer, but it purifies from yeast as a high molecular weight complex containing a 1:1 ratio of Srv2/CAP:actin [27]. In yeast extract, the molar ratio of Srv2/CAP:actin is 1:10 [27]. Mammals possess two isoforms, CAP1 and CAP2, and mammalian CAP1 is present in 1:4 molar ratio with actin in NIH3T3 and B16F1 cells [28]. This high concentration of Srv2/CAP suggests a potentially large contribution to actin dynamics *in vivo*.

The carboxy-terminal domains of all Srv2/CAPs examined bind monomeric actin [27,29–34]. Similar to cofilin and twinfilin (see below), Srv2/CAP binds preferentially to ADP–actin monomers (K_d of 0.018 μM vs. 1.9 μM for ATP–actin monomers) [35]. Unlike cofilin and twinfilin, Srv2/CAP does not inhibit nucleotide exchange from actin monomers [27,34], but actually reverses cofilin's inhibitory effect on nucleotide exchange [27]. Possibly related to this effect, low concentrations of Srv2/CAP accelerate the cofilin-mediated filament depolymerization rate [27]. Srv2/CAP also binds actin filaments indirectly through its interaction with the filament side binding protein, Abp1 [27], which might localize Srv2/CAP to regions of high actin dynamics.

One model for Srv2/CAP function is as follows: Srv2/CAP competes with cofilin for binding ADP–actin monomers [35]; Srv2/CAP-bound ADP–actin monomers exchange ADP for ATP; Srv2/CAP 'passes' its ATP–actin monomer to profilin; profilin-bound ATP–actin monomers add to filament barbed ends [35]. Thus, Srv2/CAP might accelerate actin monomer shuttling between cofilin and profilin, since profilin's affinity for ADP-bound monomers is lower than that of cofilin.

Cellular results support this hypothesis. Mammalian cells with reduced levels of CAP1 are less motile, have increased polymerized actin content, reduced actin filament turnover, and filaments that are more resistant to depolymerization by latrunculin A [28]. Similarly, yeast *srv2 capΔ* cells have reduced actin patch turnover rates [27], and profilin overexpression partially relieves the abnormal actin distribution and random budding defects seen in these cells [36]. Additionally, yeast Srv2/CAP shows genetic interactions with cofilin [27], and mammalian CAP1 colocalizes with cofilin and actin [28,34].

Aip1

Actin interacting protein 1 (Aip1) is a 67 kDa WD-repeat protein that alone has little effect on actin filament dynamics, but enhances the filament

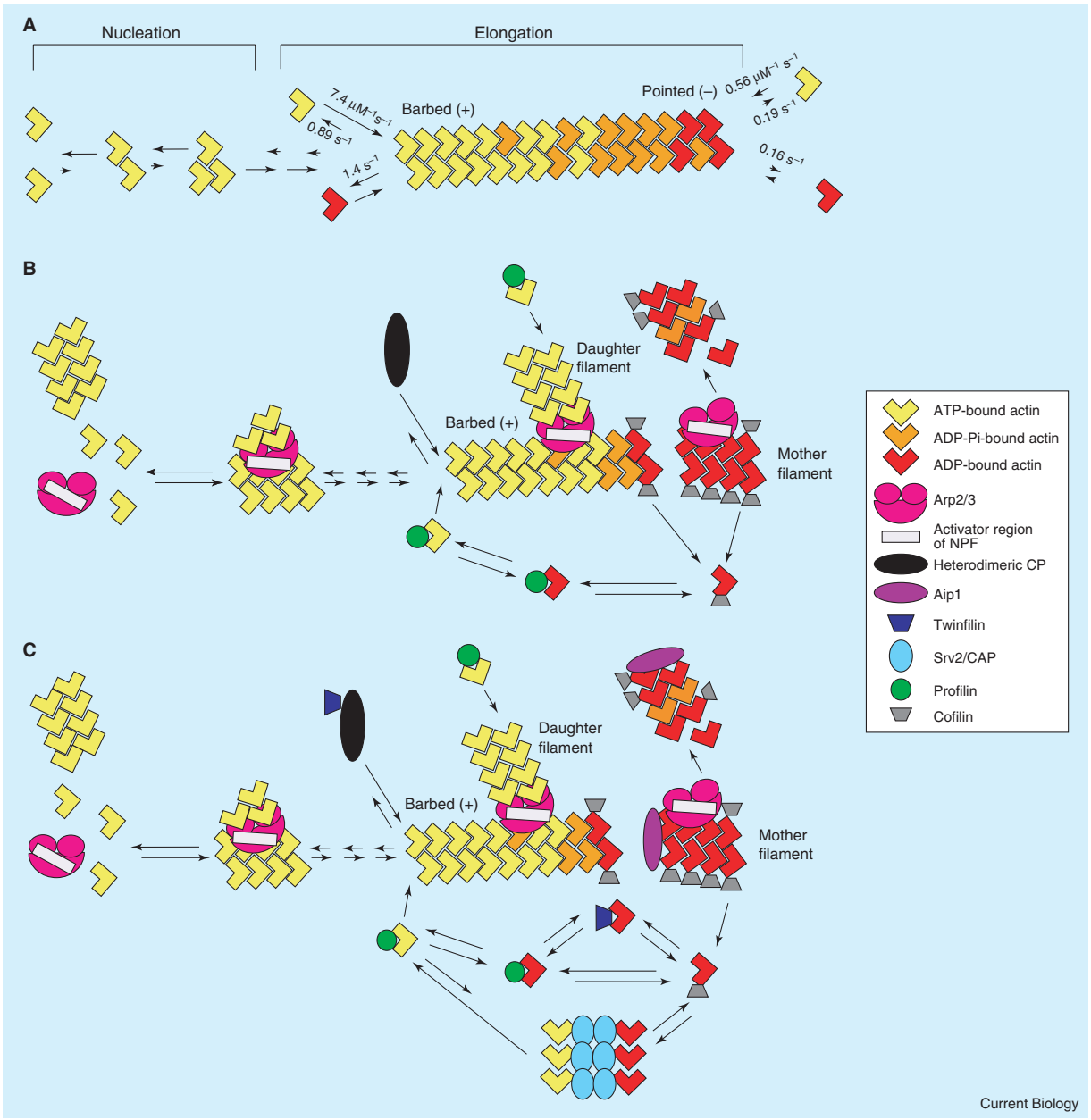

Figure 1. Actin dynamics and dendritic nucleation.

(A) Dynamics of actin alone. To assemble into filaments, actin monomers must first undergo a nucleation phase, consisting of unfavorable dimerization and trimerization reactions. Subsequent monomer addition ('elongation' phase) is favorable. Filaments are polar, with barbed (or +) ends and pointed (or –) ends. Monomers are called subunits when in filaments. Monomers can add to (and subunits can dissociate from) either end, but the barbed end is more favorable for net elongation. Monomers bind ATP tightly. Upon filament addition, monomer hydrolyzes its nucleotide slowly (about 0.3 s^{-1}), and releases the inorganic phosphate (Pi) product more slowly (about 0.002 s^{-1}). The ADP product remains tightly bound. Thus, elongating filaments contain three general sectors: barbed end, rich in ATP-bound actin subunits (yellow); the middle, rich in ADP-Pi-bound subunits (orange); and the pointed end, rich in ADP-bound subunits (red). (B) The dendritic nucleation model. Arp2/3 complex is activated by binding both an NPF and the side of a pre-existing filament. Activation allows Arp2/3 complex to nucleate a new ('daughter') filament from the side of the pre-existing ('mother') filament. Monomers add to barbed ends of both filaments, until capping by a barbed-end capping protein. Subunits hydrolyze ATP, and Pi is slowly released. Cofilin binding accelerates Pi release, triggering two events: (1) release of Arp2/3 complex from daughter filament pointed end; and (2) further cofilin binding to new ADP-bound subunits, leading to filament severing. Cofilin-bound ADP–actin subunits release from the exposed pointed (and maybe barbed) ends. Profilin competes with cofilin for these ADP-bound monomers. When profilin binds an ADP–actin monomer, it accelerates nucleotide exchange. The resulting profilin-bound ATP–actin monomer can add to uncapped barbed ends. (C) Possible accessory proteins to the dendritic nucleation model. Srv2/CAP might accelerate monomer recycling by accelerating conversion of cofilin-bound ADP–actin monomers to profilin-bound ATP–actin monomers. Aip1 caps filament barbed ends in a cofilin-dependent manner and also enhances cofilin-mediated depolymerization acceleration through an unknown mechanism. Twinfilin binds ADP–actin monomers and prevents addition to barbed or pointed ends. The significance of twinfilin's interaction with heterodimeric capping protein is unknown, but might serve to increase local monomer concentration.

disassembly rate in the presence of cofilin. Direct interaction between Aip1 and cofilin is suggested by a two-hybrid interaction for the yeast proteins [37]. In addition, Aip1 and cofilin interact genetically in both *Saccharomyces cerevisiae* and *Caenorhabditis elegans* [27,37–39]. The combination of Aip1 and cofilin reduces the barbed-end elongation rate [37,40–43]. One hypothesis is that the Aip1–cofilin complex has barbed-end capping activity [40], although Aip1–cofilin also binds filament sides at higher concentrations [37,40]. Mutagenesis studies on yeast Aip1 correlate capping defects *in vitro* with defects in cellular actin organization, suggesting that capping is an important function of Aip1 *in vivo* (K. Okada and B. Goode, personal communication). Recent evidence also suggests that, in addition to capping barbed ends, Aip1 may enhance cofilin-mediated severing [43]. In yeast, the actin:cofilin:Aip1 ratio appears to be 5:1:0.5 (B. Goode, personal communication), so that a significant amount of cofilin could be bound to Aip1. However, despite the two-hybrid interaction, attempts to demonstrate a cofilin–Aip1 interaction biochemically have been unsuccessful so far (B. Goode, personal communication).

Twinfilin

Twinfilin is a 40 kDa actin-monomer-binding protein first identified in *S. cerevisiae* [44]. Yeast has one twinfilin gene whereas mammals have two, Twinfilin1 and Twinfilin2. Despite containing two cofilin homology regions, twinfilin binds only one actin monomer [44,45]. All twinfilins examined sequester monomers and inhibit nucleotide exchange [44,46,47]. Twinfilin binds with higher affinity to ADP-bound actin (K_d = 0.05–0.12 μM) than to ATP–actin monomers (K_d = 0.47–1.96 μM) [46–48]. The high affinity for ADP–actin monomers, combined with its relative cellular abundance [46,48], implies that twinfilin could sequester an appreciable pool of monomeric actin. The situation might be more complex, however, as twinfilin can interact with heterodimeric capping protein, both *in vitro* and in cells [46,48,49], and this interaction is required for twinfilin localization to yeast actin patches [46,49]. Given that both budding yeast [44] and fission yeast [50] twinfilin knock-outs are viable and do not display any obvious phenotypes, twinfilin's actual function *in vivo* is not yet clear.

Integration of Srv2/CAP, Aip1, and Twinfilin into Dendritic Nucleation

In Figure 1C, we show how Aip1, Srv2/CAP, and twinfilin might be integrated into the dendritic nucleation model (adapted from [27]). Notably, Aip1 and Srv2/CAP may serve to enhance dramatically cofilin/profilin-mediated monomer turnover rates. Twinfilin's role as a sequestering protein may be enhanced by specific cellular localization, but its actual role in actin dynamics is unclear.

Cortactin

The reason we separate cortactin from the aforementioned proteins is that cortactin does not appear to be expressed universally by eukaryotes. Neither yeast nor *Dictyostelium* have a clear cortactin homologue, so cortactin might play specialized roles in metazoan dendritic nucleation. Another possibility is that non-metazoans possess functional cortactin homologues that have diverged significantly in primary structure.

Cortactin is a 61 kDa protein that contains an amino-terminal Arp2/3-complex-binding motif, a central actin filament-binding region, and a carboxy-terminal Src homology 3 (SH3) domain. A second isoform HS1, predominates in haematopoietic cells [51,52]. Cortactin binds Arp2/3 complex and filaments simultaneously [53], allowing cortactin to stabilize dendritic branches [54,55]. At present, it is not clear whether cortactin binds the mother or daughter filament at the branch, although the daughter filament is more likely, as affinity of this filament for Arp2/3 complex decreases 20-fold upon Pi release [56]. Thus, cortactin could stabilize this ordinarily labile association.

Cortactin colocalizes with Arp2/3 complex to the leading edge [53,57]. Interestingly, cortactin can bind and activate the NPF N-WASP independent of its ability to stabilize dendritic branches [58,59]. Thus, cortactin might have a role in both network assembly and stabilization. Cortactin is a substrate for phosphorylation by both the MAP kinase Erk and Src [57,60], suggesting that these kinases might be involved in cortactin's ability to activate N-WASP [58].

Dynamin2 is another interaction partner of cortactin, binding to cortactin's SH3 domain [61]. This interaction might explain cortactin's localization to clathrin-coated pits and its importance in receptor-mediated endocytosis [62,63]. Interestingly, both dynamin2 and cortactin localize, along with Arp2/3 complex and N-WASP, to dorsal 'waves' of membrane at the leading edge of 3T3 fibroblasts stimulated with platelet-derived growth factor. The dynamin2–cortactin interaction is necessary for the formation of these waves [64]. Since cortactin's SH3 domain also mediates its interaction with N-WASP, the dynamin interaction is probably mutually exclusive with that of N-WASP. Other conventional dynamins (dynamins 1 and 3) are also predicted to bind cortactin and, indeed, a functional dynamin3–cortactin interaction has been shown in dendritic spines [65].

Additional Nucleation Factors

Since the dendritic nucleation model was proposed, two further actin nucleation factors have been identified. At present, one hypothesis is that these factors do not act in dendritic network formation, but might assemble other actin-based structures that do not display dendritic branches, including filopodia, microvilli, yeast actin cables, and sarcomeric structures such as stress fibers and cytokinetic rings.

Formin proteins have been reviewed recently [66], so their barbed-end nucleation activity will not be discussed in detail. Nucleation is mediated by the dimeric formin homology 2 (FH2) domain. However, as discussed below, an equally important function of formins may be their ability to influence barbed-end elongation. All eukaryotes examined possess at least one formin, and most have multiple isoforms.

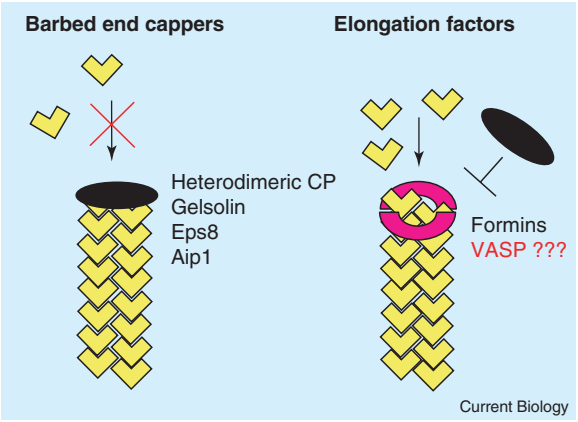

Figure 2. The battle at the barbed end.

A variety of proteins cap barbed ends, allowing no monomer addition (left). Other proteins, called elongation factors, compete with capping proteins for barbed end binding, allowing elongation even in the presence of capping protein (right). This competition, and the relative local levels of active capping proteins and elongation factors, is likely to play a major role in the architecture of specific actin-based structures.

Mammals have at least 15 formin genes [67], raising the potential for filament nucleation in a variety of cellular contexts.

Spir (sometimes called Spire), has recently been characterized [68]. Like Arp2/3 complex and formins, Spir nucleates filaments that elongate from their barbed ends. However, Spir's nucleation mechanism differs significantly from both Arp2/3 complex and formins. Four tandem WH2 repeats, which are actin-monomer-binding motifs, mediate Spir's nucleation activity. The linker sequence between the third and fourth WH2 domains is also important, and can also interact with monomer. Spir associates with pointed ends similar to Arp2/3 complex, but does not promote filament branching. Electron microscopy of Spir bound to actin reveals a novel, rod-shaped structure that corresponds to four actin monomers aligned longitudinally. This information is the basis for a nucleation model in which each Spir WH2 domain can bind a single actin monomer, stabilizing one protofilament of an actin double helix [68].

Spir appears to be restricted to metazoans, as no homologues have been identified in yeast, plants, or *Dictyostelium*. Studies in *Drosophila* show that Spir is involved in both anterior–posterior and dorsal–ventral patterning [69,70]. Interestingly, the *Drosophila* formin, cappuccino, is also important for these polarity events [69], raising the possibility that both serve to regulate the same actin structures.

Regulating Filament Elongation

In the dendritic nucleation model, capping proteins rapidly terminate barbed end elongation. A variety of capping proteins exist. Heterodimeric capping protein binds with high affinity to actin filament barbed ends (K_d = 0.1–1 nM), and is present at micromolar levels in non-muscle cells [71,72]. The gelsolin family includes at least seven members: gelsolin, villin, supervillin, advillin, capG, adseverin, and flightless I [73]. All family members tested so far cap barbed ends in a calcium-independent manner, and all but capG [74] display calcium-dependent filament severing activity. Gelsolin caps with high affinity (K_{cap} = 10 pM, [75]). The Eps8 family has recently been described as having true barbed-end capping activity (K_d = <10 nM) [76,77]. Finally, as mentioned earlier, Aip1 caps barbed ends (estimated K_d = 25 nM) in a cofilin-dependent manner [27,40].

Recent studies have shown that two protein families, formins and Ena/VASP proteins, can antagonize capping protein activity, allowing prolonged elongation even in the presence of capping proteins. Thus, formins and Ena/VASP proteins could serve as 'elongation factors' for actin filaments (Figure 2). These findings suggest the potential for exquisite regulation of filament lengths by coordinate regulation of capping proteins and elongation factors.

Six formin proteins so far have been shown to antagonize capping proteins, making it likely that this property is shared by all formins [78–81] (H. Faix, personal communication and our unpublished data). The ability of formins to antagonize capping proteins is attributed to their ability to move processively with elongating barbed ends [82–84]. While all formins block capping protein with high potency, they vary in their effect on elongation rate [66]. Profilin binding to formins increases filament elongation rate without affecting their ability to block capping protein [66].

An unanswered question is whether formin's block of capping proteins is relevant *in vivo*. Both heterodimeric capping protein and gelsolin have higher affinities for barbed ends than formins (K_d = < 1 nM vs. K_d = > 1 nM). Also, capping proteins are abundant in cells (> 1 μM), while the intracellular concentrations of formins are unknown. However, the ability of formins to remain processively attached to barbed ends after nucleation could maintain a subset of elongation-competent filaments. Indeed, a recent study shows that the fate of a barbed end relies on which protein (formin or capping protein) binds first [50].

The role of Ena/VASP proteins in barbed end dynamics is not as clear as for formins. Several lines of evidence suggest 'anti-capping' functions for mammalian Ena/VASP. Cellular studies show localization of Ena/VASP to the leading edge plasma membrane and to filopodial tips [85–88]. This localization is disrupted by treatment with cytochalasin D [89]. Biochemical assays show that VASP antagonizes capping protein activity [89] (M. Barzik and D.A. Schafer, personal communication). However, others find no evidence for competition between mammalian Ena/VASP and capping proteins [90]. Additionally, *Dictyostelium* VASP does not antagonize capping protein, but does interact with the formin dDia2 (H. Faix, personal communication). Yeast do not have a VASP homologue.

A major question is how capping proteins and elongation factors can be regulated coordinately. Polyphosphoinositides inhibit capping by heterodimeric capping protein and gelsolin [91–93]. Eps8 may be autoinhibited, and require activation by Abi1 [76]. Aip1 exerts its influence on actin filament dynamics only in the presence of cofilin [24,40–43]. Some

formins are auto-inhibited, and require activation by Rho GTPases (reviewed in [66]). VASP is regulated by phosphorylation, and by binding to proteins that influence its localization [94]. Thus, localized activation of cappers or elongation factors may play a role in controlling which filaments can elongate and which cannot. An intriguing result, as yet unexplained, is that actin filaments generated from GTP–Cdc42-treated neutrophil extracts grow faster than expected for free actin filaments [95]. Could this be a case of elongation regulation, possibly involving a formin?

Cofilin as a Filament Generator

While generally considered a filament depolymerization factor, cofilin also can stimulate filament assembly under some circumstances. In metastatic mammary adenocarcinoma cells, epidermal growth factor (EGF) stimulation causes rapid formation of a dendritic network at the periphery [13]. Three different experimental approaches suggest that cofilin activity is necessary *before* Arp2/3 complex for filament assembly in this system [14,96,97]. A model to explain this activity proposes that severing by cofilin creates new barbed ends that elongate (reviewed in [98]). These newly elongated filaments are preferred as Arp2/3 complex activators over older filaments [99], and trigger Arp2/3-complex-mediated dendritic nucleation. A similar model has been proposed for the action of gelsolin in platelets [100].

In this model, cofilin-mediated filament generation is required for initiation of dendritic nucleation, but may be dispensable thereafter, since Arp2/3 complex can then nucleate subsequent new filaments for continued activation. Thus, cofilin might serve two roles: to initiate dendritic nucleation; and to accelerate depolymerization during monomer recycling. The initiator activity might be particularly important for processes requiring directional changes and 'stop-start' motility, such as neuronal pathfinding and chemotaxis.

Immunolocalization studies support these dual roles for cofilin. In the EGF-stimulated system, cofilin enriches more rapidly than Arp2/3 complex to the leading edge, the site of dendritic network formation [15]. In slowly migrating fibroblasts, cofilin also localizes to the leading edge [12], and this localization is specific for the unphosphorylated, active form [101]. However, in rapidly migrating fish keratocytes, cofilin localizes slightly behind Arp2/3 complex [12], which might suggest that its recycling role predominates in this case.

Yeast Actin Patches — Multiple Phases of Dendritic Nucleation?

Actin patches are small (<0.5 μm diameter), short-lived (5–20 s half-life), and rapidly moving (approximately 0.5 μm s^{-1}) regions of high actin filament concentration in yeast [102–106]. Growing evidence suggests that patches are sites of endocytosis [106,107]. Arp2/3 complex localizes to patches [108] and is required both for patch assembly and motility [109]. Furthermore, actin filaments in patches possess dendritic branches characteristic of Arp2/3 complex [23,24].

Budding yeast has multiple Arp2/3 complex NPFs, including Pan1p, Abp1p, Las17p, and the myosin I proteins, Myo3p and Myo5p. Live-cell imaging studies reveal an intricate temporal sequence by which these NPFs and Arp2/3 complex localize to patches [107,110]. Superimposed on this pattern are changes in patch motility, from very little motility as initial components (including Pan1p and Las17p) assemble on the plasma membrane, to slow motility away from the plasma membrane as other components (including Abp1, Arp2/3 complex, and actin filaments) accumulate, to rapid motility toward the cell interior as initial components (Pan1p, Las17p) release [107]. Myo5p localizes to the patch only briefly, just prior to the rapid motility phase [110]. The filament architecture at specific times in patch lifespan is not known.

Thus, patch dynamics might represent a modified version of dendritic nucleation, with different NPFs activating Arp2/3 complex at different phases to affect different functions. Given these modifications, one wonders about the roles of other dendritic nucleation proteins in patch dynamics. Cofilin and capping protein enrich in patches (reviewed in [111]). Perturbation of cofilin function [112] was found not to affect overall patch motility, but these observations were made before the different stages of patch motility were fully appreciated. Loss of capping protein has no effect on the speed of actin patches in measurements of short-range movements over small time periods [113]. However, longer-range movements, observed over longer time periods, are inhibited (K. Kim, B. Galetta and J. Cooper, personal communication). Further investigation should reveal the roles of these proteins during specific patch motility phases.

Dendritic Nucleation in a Migrating Cell

Many eukaryotic cells migrating on a substratum display a characteristic motility sequence, including: protrusion of their 'leading edge' membrane; adhesion of the newly protruded region to the substratum; advancement of the cell body; and retraction of the 'trailing edge' (reviewed in [114,115]). Leading edge protrusion is thought to be driven by actin polymerization, while advance of the cell body (the bulk of the cell, including the nucleus) is driven by actomyosin-based contraction. A generic model of a 'typical' motile cell is shown in Figure 3 (please see figure legend for qualifications). In fact, the model cell systems used by investigators can differ significantly in terms of both the rate and the persistence of protrusion. As two examples, fibroblasts exhibit slow (around 1 μm/min) and intermittent movement, whereas keratocytes exhibit rapid (>10 μm/min) and persistent movement. Neuronal growth cones have additional differences, as outlined later. The data discussed below are derived from studies of multiple cell types, and some of the current discrepancies in the field might be due to differences in motility mechanisms between these cells.

In the remainder of this review, we discuss the actin cytoskeleton in what we refer to as the 'protrusive region' of a migrating cell. In other words, the area involved in the first stage of motility. The dendritic

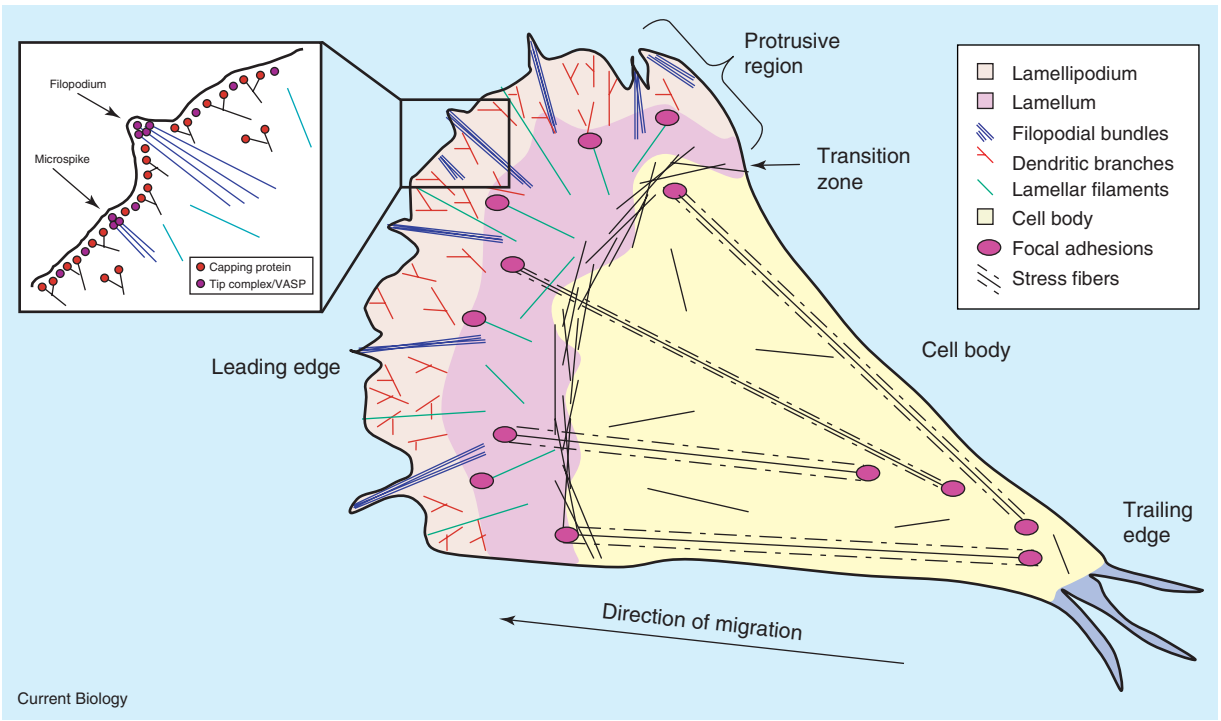

Figure 3. Model of a 'typical' motile cell.

In reality, there is no such thing as 'typical'. However, we feel that this model, which is based most closely on a fibroblast, illustrates many of the actin-based structures involved in motility. Several qualifications must be made. First, the model is not to scale, as the lamellum is typically much wider than the lamellipodium. Second, the origin of filopodia and microspikes (from lamellipodium or lamellum) is not definitively known. Third, the morphology of lamellar filaments (bundles of long filaments, bundles of short filaments, parallel or perpendicular to direction of motility) is not definitively known, and may vary by cell type. Fourth, the transition zone contains dense actin filaments (and myosin II) oriented perpendicularly to the direction of motility, and may be derived from re-orientation of lamellar filaments, but this relationship is not definitively known. Fifth, some of the actin-based structures depicted are not present in all motile cells. For example, fish keratocytes do not have filopodia, microspikes, or stress fibers. Sixth, stress fibers encompass a heterogeneous group of actomyosin contractile structures (see end of the review). The inset on the left highlights an expanded region of the leading edge, showing capping protein exclusion from tips of filopodia and microspikes. N.B. Regarding nomenclature, we have taken note of the correct Latin grammar, so 'lamellipodium' and 'lamellum' are singular, and 'lamellipodia' and 'lamella' are plural, with the adjectival forms being 'lamellipodial' and 'lamellar'. Similar nomenclature applies for filopodia (filopodium, filopodia, filipodial).

nucleation model was developed partly to explain actin dynamics in this region. Recent work has shown, however, that the protrusive region is more complex than described by the dendritic nucleation model alone.

Lamella and Lamellipodia in the Protrusive Region of a Migrating Cell

Early observations of motile cells recognized that two morphologically distinct actin networks, called lamellipodium and lamellum (see note at end of Figure 3 legend for nomenclature), were present in the protrusive region of a migrating cell (Figure 3) [11,116]. The lamellipodium, at the leading edge, is the site of rapid membrane protrusion and retraction and is composed primarily of a dendritic network. The lamellum, spatially located between the lamellipodium and the cell body, is instead composed of bundles of long actin filaments. While the lamellipodium contains abundant Arp2/3 complex, capping protein and cofilin [12–17], the lamellum is rich in tropomyosin and myosin II [11,117].

Quantitative fluorescent speckle microscopy (qFSM) has recently emerged as a powerful tool for analyzing molecular dynamics in live cells, and several studies have used qFSM to examine actin dynamics in the protrusive region [118–122]. Key to these studies is the ability to quantify multiple parameters independently for fluorescent actin speckles, including movement rates and lifetimes for single speckles or groups of speckles. Through this work, four key characteristics have been established for distinguishing between lamellipodial and lamellar networks — actin flow rates, mechanisms of actin network movement (kinematic signatures), actin turnover rates (kinetic signature), and key molecular components (molecular signature) [121,122].

While the lamellipodium exhibits rapid actin flow rates (300–500 nm/min), as well as bands of rapid polymerization and depolymerization parallel to the leading edge, the lamellum exhibits slower flow rates (100–250 nm/min), with individual puncta of polymerization and depolymerization (Figure 4). Strikingly, 85–90% of the filaments generated in the lamellipodium depolymerize at or before the lamellipodium–lamellum transition zone, indicating that the lamellipodial and lamellar filaments are independent entities. In other words, the filaments formed in the

lamellipodium are not simply carried backward, by retrograde flow, to form the filament bundles of the lamellar network [121].

More detailed analysis reveals two general classes of actin speckles — fast-moving and short-lived (class I), and slow-moving and long-lived (class II). Class I speckles localize primarily to the lamellipodium and display a lamellipodial kinetic signature, whereas the majority of the class II speckles localize to the lamellum and appear lamellar kinetically (Figure 4). However, nearly one third of the lamellipodial speckles are class II, and class II speckles can be found at the extreme leading edge, suggesting that lamellipodial and lamellar networks overlap [121].

Additional studies suggest that the lamellar network alone can drive persistent cell protrusion [122]. Microinjection of skeletal muscle tropomyosin results in loss of the leading edge lamellipodium and expansion of the lamellum. This change might be caused by tropomyosin's known inhibitory effects on Arp2/3 complex [123] and cofilin [117] activities. Notably, injected cells exhibit longer persistence of leading edge protrusion, which in turn yields an increase in rates of cell advancement over those of control cells.

Thus, our image of the leading edge is being revised (Figure 4). The lamellipodial network at the extreme leading edge appears to be driven by dendritic nucleation. However, this network terminates abruptly 1–3 μm from the leading edge, after which the lamellar network predominates. This suggests that the lamellar network is not formed by remodeling of the lamellipodial network, but is assembled independently. Techniques that allow single filament resolution in this region are necessary to support this conclusion. Additionally, a substantial percentage of lamellar filaments assembles within the lamellipodial region. Finally, the lamellar network alone may be sufficient to drive leading edge protrusion.

These findings raise a number of intriguing questions. Are lamellipodia and lamella redundant in their role of pushing the leading edge forward, or do lamellipodia serve another function, such as pathfinding? Another unresolved issue concerns the mechanism of lamellar filament assembly. If Arp2/3 complex is not the nucleator for these filaments, what is? One possibility is that specific members of the formin family nucleate these filaments [82].

The roles of non-muscle tropomyosin isoforms in cell motility are also unclear at this point. In muscle sarcomeres, tropomyosin on thin filaments inhibits myosin interaction [124]. Since both tropomyosin and non-muscle myosin II are abundant in the lamellum, does tropomyosin play a similar role here? Or, could non-muscle tropomyosin isoforms have different effects on the activity of non-muscle myosin II, or on other non-muscle myosins? Alternatively, is tropomyosin's main function to block Arp2/3 complex and cofilin activity in the lamellum? For that matter, what is the significance of the multiple non-muscle tropomyosin isoforms [125], and do these isoforms have specific subcellular functions?

Filopodia and Lamellipodia at the Leading Edge

Protrusive filopodia are present at the leading edge of some, but not all, motile cells. For example, nerve growth cones and most fibroblast lines possess filopodia, but fish keratocytes and mammalian neutrophils do not. Nomenclature heterogeneity exists for filopodia, as the name 'microspike' is also used for these structures. We employ a nomenclature described in [126], whereby filopodia project beyond the lamellipodial edge, while microspikes do not (see inset in Figure 3).

Filopodia are morphologically distinct from dendritic networks. In contrast to the branched filament network of the lamellipodium, filopodia are composed of long, bundled, unbranched actin filaments. Neither Arp2/3 complex nor cofilin are present in filopodia [12]. Platinum replica electron micrographs demonstrate that fibroblast filopodia are rooted in the lamellipodium/lamellum [12,88]. Similarly, nerve growth cone filopodia extend deeply into the growth cone [127,128], and their constituent actin filaments are extremely long-lived compared to those in the surrounding network (half-lives of 25 and <3 min, respectively [129]). Filopodia alternate between growth and shrinking, controlled by a balance between barbed end monomer addition and retrograde flow [129].

How do leading edge filopodia assemble? A 'convergent elongation' model was recently proposed, which extends the dendritic nucleation model to accommodate filopodial formation and elongation [17,88,130]. In the model, Ena/VASP and capping protein compete for free barbed ends of nascent Arp2/3-complex-generated filaments. Filaments that associate with capping protein stop elongating, while Ena/VASP-bound filaments continue to grow (Figure 3 inset). By an unknown mechanism, these Ena/VASP-associated filaments lose their dendritic morphology, and assemble into parallel bundles through the crosslinking protein, fascin. VASP remains at the barbed end of the elongating filopodium, and is part of an electron-dense 'tip complex'.

A series of cellular studies supports this model [17,88,130]. In addition, studies conducted *in vitro* support the hypothesis that fascin-dependent bundles are initiated by Arp2/3 complex [130]. Other studies argue against filopodial initiation by Arp2/3 complex [131]. Expression of a construct that inhibits Arp2/3 complex activity blocks lamellipodial assembly in two cell types [122,131]. However, expression of this construct does not perturb filopodia in fibroblasts or in nerve growth cones [131]. In fact, Arp2/3 complex inhibition (by inhibitory construct expression or skeletal muscle tropomyosin microinjection) causes formation of filopodia-like structures in PtK1 cells, which ordinarily lack filopodia [122].

Thus, the mechanism of filopodial assembly is still in dispute. Could this be due to redundancy in filopodial assembly mechanisms? Could filopodia emanate from lamellar, rather than lamellipodial, filaments? Regardless of how these structures form, control of elongation plays a major role in regulating their dynamics. This regulation is exquisitely precise, as elongation and retraction of all filaments in the

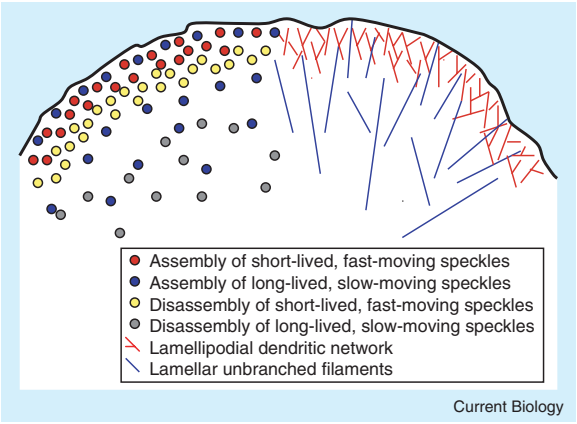

Figure 4. Lamellipodia and lamella in the protrusive region.

The lamellipodial network is assembled by dendritic nucleation at the extreme leading edge, and disassembles in a narrow region 1–3 μm behind the leading edge. Lamellar filaments display puncta of assembly throughout the protrusive region, but the density of these puncta increases with distance from the leading edge. Lamellar filament disassembly also occurs throughout the protrusive region, with higher density of disassembly toward the rear. Thus, lamellipodial and lamellar filaments overlap in distribution, and are assembled and disassembled independently.

filopodial bundle is coordinately controlled [129]. Clearly, Ena/VASP proteins and others in the barbed-end tip complex play a major role in this control [17,88]. The formin protein mDia2 also appears to play a role in filopodial dynamics and localizes to filopodial tips [132,133], and its ability to modify elongation rates (reviewed in [66]) might be important in filopodial regulation. Myosin X is also enriched at filopodial tips, and its expression level correlates with filopodial length [134].

It should be noted that there are significant differences between growth cones and protrusive regions of other cells. The actin network in growth cones appears to contain more long filaments and less apparent dendritic branches [128,131], and Arp2/3 complex is not concentrated at the leading edge [131]. In addition, while Arp2/3 complex inhibition causes major changes in fibroblast lamellipodial morphology [122,131], analogous changes are not apparent in growth cones [131]. The nature of these differences requires further study.

Conclusions and Future Directions

One valuable feature of the dendritic nucleation model is that it has provided focus for a large number of subsequent biochemical and cellular studies. These studies have broadly supported the model, while revealing potential modifications. Additional proteins, such as Srv2/CAP, Aip1, twinfilin, and cortactin, might have roles in dendritic nucleation. Cofilin appears to act in nucleation initiation, in addition to its role in network disassembly. Elongation factors might modify network structure, and allow transition to filopodial bundles. At the leading edge, a second lamellar actin-based network co-exists with the lamellipodial dendritic network, with independent assembly and disassembly mechanisms.

In closing, we would like to point out two other cellular actin-based structures that might be independent of dendritic nucleation. The first are microvilli, which encompass a variety of finger-like, actin-bundle-containing protrusions that are not attached to a substratum. Included in this category are stereocilia, *Drosophila* bristles, epithelial brush border microvilli, and short, dynamic protrusive structures on lymphocytes and other cells [135–137]. As opposed to most filopodia, microvilli are often not associated with a clear lamellipodium or lamellum, although more detailed analysis might reveal such an association. While a number of studies have greatly advanced our understanding of the molecules controlling microvillar dynamics [138–141], assembly mechanisms remain obscure.

Another group of actin-based structures falls largely under the popular name ‘stress fibers’, with the common theme being that all are assemblies of actin filaments and non-muscle myosin II. This group is highly heterogeneous in actin/myosin architecture, including alternating polarity bundles, mixed polarity bundles, and graded polarity bundles [142–145]. Cytokinetic rings also fall into this category. Where tested, these structures appear contractile [146,147]. Assembly mechanisms are unclear, although formins appear to play a role in cytokinetic ring assembly [148–150]. Many transfection studies in mammalian cells claim to induce ‘stress fibers’ without clearly showing that these structures contain myosin II, a practice that should be strongly discouraged.

Thus, the dendritic nucleation model has pushed the field forward and, in doing so, has exposed further mysteries. The continued generation of detailed, testable models based on biochemical properties will allow for future progress.

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