

# Control of actin assembly and disassembly at filament ends

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The most important discovery in the field is that the Arp2/3 complex nucleates assembly of actin filaments with free barbed ends. Arp2/3 also binds the sides of actin filaments to create a branched network. Arp2/3's nucleation activity is stimulated by WASP family proteins, some of which mediate signaling from small G-proteins. *Listeria* movement caused by actin polymerization can be reconstituted *in vitro* using purified proteins: Arp2/3 complex, capping protein, actin depolymerizing factor/cofilin, and actin. actin depolymerizing factor/cofilin increases the rate at which actin subunits leave pointed ends, and capping protein caps barbed ends.

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## Abbreviations

**ADF** actin depolymerizing factor

**Aip1** actin-interacting protein-1

## Introduction

Actin filaments grow or shrink by virtue of the addition or loss, respectively, of actin subunits from either end of the filament. The two ends, barbed and pointed, are both able to add and lose subunits. Polymerization is favored at the barbed end over the pointed end in terms of steady-state binding affinity. Kinetic rate constants for both polymerization and depolymerization are greater at the barbed end.

Proteins that bind filament ends control actin polymerization. First, they cap filament ends, such that subunits cannot be added or removed. Second, some can nucleate the formation of new filaments, which is otherwise unfavorable. A number of end-binding proteins have been defined biochemically. How they function *in vitro* and *in vivo* is a current focus of the field.

Proteins that bind the sides of actin filaments can also influence how fast subunits leave the filament. Proteins, such as tropomyosin, which bind to multiple subunits along the side of a filament and proteins that crosslink filaments decrease the rate of subunit loss from ends. An exciting discovery is that ADF/cofilin proteins increase the rate of subunit loss from pointed ends.

*In vivo*, these proteins work simultaneously to produce motility. Understanding how their individual actions on actin are coordinated is an essential goal, thus the field has been investigating how combinations of actin-binding

proteins affect actin polymerization. One exciting example is the discovery that three actin-binding proteins, plus actin, are sufficient for *Listeria* to induce actin polymerization and motility [1••].

This field has been the subject of a number of reviews in recent years. We reviewed this topic in more depth several years ago [2]. More recent reviews have addressed filament length in muscle [3], actin polymerization dynamics and signaling [4], how WASP links small G proteins to Arp2/3 complex [5], how Arp2/3 complex functions [6–8], how ADF/cofilin proteins function [9,10] and how gelsolin and its relatives function [11]. In this minireview, we focus on the most recent advances in understanding how actin-binding proteins control the addition and loss of subunits at filament ends.

## Barbed ends: capping protein (Cap Z)

When cells induce actin polymerization, especially to cause movement at the cell periphery, free barbed ends are created and add subunits. Over time, capping protein binds barbed ends to stop filament growth. The cessation of filament growth by capping protein may be necessary for polymerization to occur at specific times and places *in vivo* (i.e. free barbed ends probably exist only when they are newly created or when capping protein is inhibited in their vicinity). Since nearly all barbed ends are capped by capping protein, polymerization will be confined or 'funneled' to the free barbed ends. This hypothesis for localized actin assembly, proposed by Carlier and Pantaloni [12], has received experimental support with the discovery that capping protein is necessary for actin polymerization and motility of *Listeria* in a system comprising pure proteins [1••]. In those experiments, as the concentration of added capping protein was decreased to zero, *Listeria* motility fell to zero, which is consistent with the idea of 'funneling'. In support of this hypothesis *in vivo*, capping protein is a component of the actin filament tails whose polymerization drives the movement of *Listeria* [13]. The model also can explain the observation in *Dictyostelium* that loss of capping protein led to increased F-actin but decreased motility [14].

Actin filaments with capping protein at their barbed ends can be uncapped by polyphosphoinositides *in vitro* [15], and this may occur during platelet activation [16]. However, uncapping appeared not to occur during chemoattractant-induced actin assembly in *Dictyostelium*; capping protein only terminated polymerization [17].

Some studies suggest that capping protein may be inhibited or may be unable to cap barbed ends under certain conditions *in vivo*. Barbed ends of red cell membrane preparations seemed to be resistant to capping protein

[18]; barbed ends in red cells may be capped by adducin instead [19]. Also, barbed ends induced in neutrophil extracts by Cdc42 appeared to be protected from capping protein [20\*]. Perhaps some factor inhibits capping protein or prevents it from binding in these cases.

In striated muscle, capping protein binds to barbed ends of actin filaments at the Z line, the inspiration for the name 'capZ.' This topic was recently reviewed [3]. In new work consistent with this view, expression of a mutant capping protein unable to bind actin led to severe sarcomere disruption in hearts of transgenic mice [21]. In addition, expression of a nonsarcomeric isoform of capping protein (beta2) had the same severe effect on sarcomere assembly, indicating that the nonsarcomeric isoform cannot substitute for the sarcomeric isoform, beta1 [20\*].

### Gelsolin and its superfamily

Gelsolin, which was thoughtfully reviewed in *Current Opinion in Cell Biology* last year [11], appears to play an important role in certain situations where actin assembly is induced. In most cells at rest, gelsolin is not bound to actin. However, stimulation, especially with increased  $Ca^{2+}$  or  $H^+$ , can cause gelsolin to sever and cap actin filaments. If gelsolin-capped filaments can be uncapped to create free barbed ends, polymerization may be induced. Activation of heterotrimeric and small G proteins dissociated gelsolin from barbed ends [22]. Fibroblasts from gelsolin knockout mice were defective for formation of lamellipodia in response to Rac signaling [23], which depends on actin polymerization.

Depletion or inhibition of gelsolin in cultured cells led to loss of stress fibers and contractility [24]; in contrast, fibroblasts from gelsolin-knockout mice had increased F-actin in stress fibers [23]. These observations confirm a role for gelsolin in remodeling the actin cytoskeleton but reveal the potential complexity of gelsolin's effects on actin assembly *in vivo*.

During apoptosis, gelsolin is proteolyzed by caspase-3 to a form that no longer needs  $Ca^{2+}$  for activity. This active fragment of gelsolin contributes to progression of apoptosis, presumably through severing actin filaments [11].

Villin, which is related to gelsolin, has a small headpiece that bundles actin filaments *in vitro*, in addition to the severing and capping activities of the gelsolin-related portion of the protein. This bundling activity of villin is clearly able to function *in vivo* as seen in transfection experiments in cultured cells [25] and overexpression in *Drosophila* oocytes [26], but whether the bundling activity of endogenous villin is an important element of its function *in vivo* remains unclear. Notably, in a villin knockout mouse, the assembly of actin bundles in microvilli of intestinal epithelial cells, which normally contain villin, was normal [27,28\*]. However, microvilli also have other bundling proteins, and the intestinal lining expresses a villin relative,

advillin, whose function may overlap with that of villin and thereby mitigate the severity of the phenotype in the villin knockout [29]. Another new relative, albeit more distant, which also bundles actin filaments at the plasma membrane of villin is supervillin [30].

On the other hand, the severing activity of villin may have a more essential role *in vivo*. Brush borders from intestinal epithelial cells of a villin knockout mouse did not disassemble their actin bundles in response to high  $Ca^{2+}$  concentrations [28\*]. Moreover, *in vivo*, in an experimental model for intestinal epithelial injury that includes loss of filamentous actin from the brush border, a villin-knockout mouse showed decreased loss of actin, increased severity of epithelia injury and greater probability of death [28\*].

### Pointed ends: Arp2/3 complex

Several exciting discoveries about Arp2/3 complex have now given it a central role in our view of how actin polymerization occurs in cells. Purified Arp2/3 complex binds pointed ends and nucleates the formation of actin filaments with free barbed ends [31\*\*]. Creation of free barbed ends has been up until now a poorly understood feature of actin polymerization in cells. Arp2/3 simultaneously binds to the sides of actin filaments, creating a branching network of filaments [31\*\*]. The filament network created *in vitro* is very similar to the actin filament network observed *in vivo* in the cortex of migrating cells. In the cortex, Arp2/3 was localized to branch points where pointed ends meet the sides of filaments [32\*\*]; this observation provides a major piece of evidence that the biochemical model is correct *in vivo*. In addition, in studies localizing actin polymerization in living cells, Arp2/3 was localized at sites of actin polymerization and motility [33\*]. Arp2/3 was also found in the zone of actin polymerization and motility at the leading edge of lamellopodia [32\*\*,34,35].

Another exciting discovery is that the nucleating activity of Arp2/3 is greatly stimulated by signaling proteins that are implicated in inducing actin polymerization. WASP and its relative Scar1 bind directly to Arp2/3 [36], which greatly increases Arp2/3's nucleation activity [37\*]. WASP-coated beads placed in a cell extract polymerized actin and moved, which required Arp2/3 [38\*]. In cell extracts, actin polymerization can be stimulated by Cdc42, which also required Arp2/3 complex [39,40]. N-WASP appeared to mediate the interaction between Cdc42 and Arp2/3 [41\*].

In *Listeria*-induced actin polymerization and movement, the *Listeria* protein ActA interacts with Arp2/3 complex and increases its actin nucleation activity [42\*]. This interaction is predicted to be necessary for the bacterial motility caused by actin polymerization in host cell cytoplasm. The presence of Arp2/3 was necessary for *Listeria* motility [43]. Also, Arp2/3 was one of a minimal set of three actin-binding proteins sufficient to reconstitute the actin polymerization and movement associated with *Listeria* [1\*\*].

In yeast, the WASP relative Bee1/Las17 also stimulates Arp2/3 biochemically [44]; however, a truncated form of Bee1 that lacks a region necessary for Arp2/3 binding functions normally *in vivo*, showing that the Bee1–Arp2/3 interaction is not necessary *in vivo* [44]. Arp2/3 mutants show loss of actin patch movement [45], but this movement appears not to depend on actin assembly [46,47]. Therefore, the roles of Arp2/3 in yeast and animal cells may be different.

### Tropomodulin

Tropomodulin caps the pointed ends of the actin-based thin filaments in striated muscle, playing an essential role in the assembly of the sarcomere. Tropomodulin also binds tropomyosin, which greatly increases its affinity as a pointed end cap [48]. This topic was recently reviewed [3]. In newer work, the distribution of tropomodulin early in myofibrillogenesis suggests that pointed ends of actin filaments are capped early. Thus, whole actin filaments, with both ends capped, may slide into place as the sarcomere matures, as opposed to filaments polymerizing and depolymerizing at their ends [49].

The function of tropomodulin in nonmuscle cells is not understood well. In *Drosophila*, the tropomodulin homologue sanpodo is necessary for Notch-based signaling of cell fate in the peripheral nervous system [50,51] and muscle [52]. The affected cells have an altered actin distribution. Most likely, actin is necessary for asymmetric localization of cell fate determinants, and the loss of sanpodo/tropomodulin impairs the assembly and function of the actin cytoskeleton. In vertebrates, tropomodulin has been described as part of the actin cytoskeleton in erythrocyte membranes and eye lens cells [53].

### Side-binding proteins

Many proteins that bind to the sides of actin filaments are polyvalent and thus stabilize filaments by inhibiting the loss of subunits from ends. Such proteins include tropomyosin, filament bundlers and filament crosslinkers.

### ADF/cofilin

The actin dynamics that underlie cell motility and morphogenesis require the disassembly of actin filaments as well as their assembly. Proteins of the ADF/cofilin family, which includes ADF, cofilin, actophorin, depactin and destrin, mediate actin filament disassembly [9,10]. Actin filaments shorten in the presence of ADF/cofilin proteins, which could occur in two ways: by severing, thereby creating more filament ends that disassemble; and by increasing the rate of subunit loss from filament ends.

Strong evidence for the existence of severing came from films of fluorescent actin filaments treated with ADF/cofilin, in which single long filaments clearly broke into smaller pieces [54]. Additional evidence supporting severing includes analysis of the kinetics of actin assembly in the presence of cofilin [55,56,57]. These data were best fit by

a severing mechanism, based on kinetic modeling. Severing is more likely to occur when filaments are long. ADF/cofilin had little severing activity on short filaments prepared by capping with gelsolin [55,58,59].

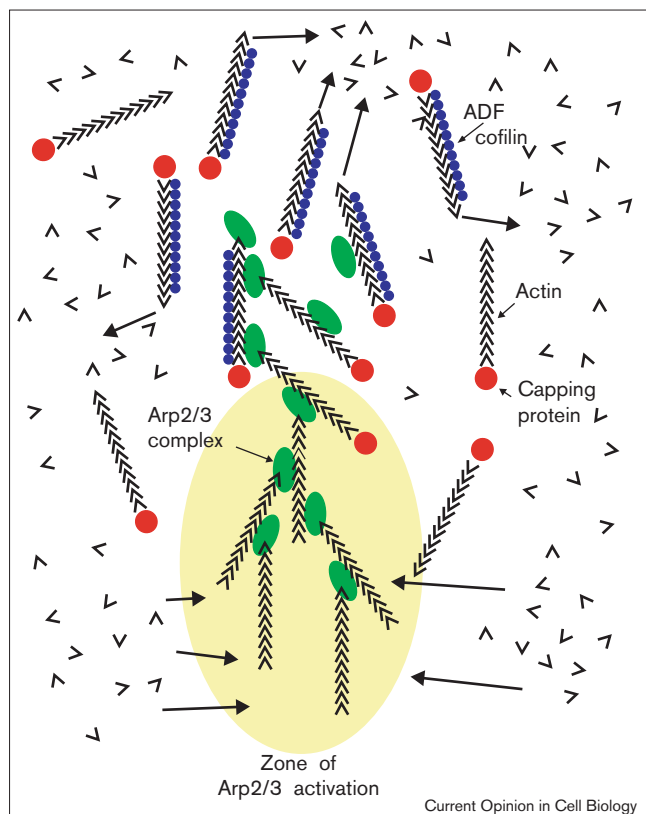
On the other hand, Carlier *et al.* [58] observed a 25-fold increase in the turnover of F-actin in the presence of ADF/cofilin that could not be accounted for solely by severing. They proposed an alternative mechanism whereby ADF/cofilin accelerates the rate-limiting step in filament disassembly by increasing the off-rate of actin subunits from pointed ends. This view was supported by analyses of the effect of ADF/cofilin on actin disassembly when barbed ends were capped [55,58]. This ability of ADF/cofilin to increase F-actin turnover can account for the level of actin assembly/disassembly required for the rates of cell motility observed *in vivo* [58]. As predicted using this model, Arp2/3 complex binding to pointed ends decreased but did not eliminate the effect of ADF/cofilin on F-actin turnover [59].

One complexity in the field is that effects of ADF/cofilin proteins on actin vary considerably depending on the source of the ADF/cofilin protein, as well as on the source of the actin and conditions of ionic strength and pH. Despite these difficulties, the field is approaching a consensus view that both filament severing and increased pointed-end subunit loss occur [9,60]. A quantitative understanding of the relative contributions of the two mechanisms, especially *in vivo*, is a current challenge for the field.

Structural analysis of ADF/cofilin binding to actin filaments has provided important information about how it affects filament dynamics [61]. ADF/cofilin bound cooperatively along the side of an actin filament, at a 1:1 ratio. ADF/cofilin binding changed the orientation of actin subunits within the filament, resulting in a change in the twist of the filament. This change in filament architecture may alter subunit contacts and weaken the lateral and longitudinal interactions between subunits, making the filament more likely to break and subunits more likely to dissociate from ends [62]. In addition, the altered filament structure induced by ADF/cofilin may induce the dissociation of pointed end-binding proteins, such as Arp 2/3 complex, leading to filament disassembly.

Small GTPases induce actin dynamics in cells by stimulating factors that promote actin assembly and by inhibiting factors that promote its disassembly. Several new observations indicate that ADF/cofilins are regulated downstream of the Rho family of GTPases. It has long been appreciated that phosphorylation of ADF/cofilin at a specific serine residue abolishes its actin-binding activity [10]. LIM kinase was identified as the kinase that phosphorylated cofilin at this serine residue *in vitro* and *in vivo*, where its ability to phosphorylate ADF/cofilin was linked to the activation of the small GTPase, Rac [63,64]. The ability of LIM kinase to

Figure 1



A current model for stimulation of actin polymerization, illustrating flux through a system not at equilibrium. The polymerization of actin (black chevrons) is activated within a zone of signals (yellow oval) that activate Arp2/3 complex (green oval) and may also inhibit capping protein (red circle). Arp2/3 complex binds to sides of actin filaments, which also increases its nucleation activity. Filaments nucleated by Arp2/3 have their pointed ends capped and their barbed ends free. Filaments grow by addition of subunits to the free barbed ends. After some time, the barbed ends become capped by capping protein, which stops their growth. The lifetime of the free barbed ends depends simply on the time of the association reaction with capping protein. However, if capping protein is inhibited in the active zone, however, barbed ends will only become capped when they leave the active zone, perhaps moved by the force of polymerization. ADF/cofilin (blue circle) binds cooperatively and preferentially to older actin filaments that contain ADP. This increases the dissociation rate of actin subunits from pointed ends. To lose subunits, pointed ends need to be uncapped, losing Arp2/3 complex. Arp2/3 dissociates at its normal rate or at an increased rate owing to the altered filament structure caused by ADF/cofilin. The subunits lost from the pointed end diffuse and are available to add to new free barbed ends. Thus, actin polymerization is 'funneled' to the active zone because free barbed ends are created there and because older barbed ends are capped by capping protein.

phosphorylate ADF/cofilin also is regulated by phosphorylation via Pak1 [65<sup>\*</sup>], an effector of Rac, and via Rho-associated kinase (ROCK) [66<sup>\*</sup>], an effector of Rho. These multiple pathways for ADF/cofilin regulation may contribute to the spatial and temporal specificity of regulation of actin assembly associated with various cells and signals.

Overexpression of LIM kinase, which should inhibit cofilin, blocked actin assembly and motility at the leading edge

of the cell and at sites within the lamella [33<sup>\*</sup>]. This result is consistent with the hypothesis that ADF/cofilin promotes actin depolymerization globally, which is necessary for localized polymerization. This view is also supported by the observation that ADF/cofilin is found only in the proximal portion of the cortex and not at the distal edge where polymerization occurs [32<sup>\*\*</sup>]. On the other hand, the actin filaments in the ADF/cofilin-containing region of the cortex were stable during a cell extraction procedure that caused depolymerization in general [32<sup>\*\*</sup>]. Therefore, extracted factors may be necessary for ADF/cofilin to promote disassembly.

A second protein regulator of ADF/cofilin increases its actin disassembly activity. *In vitro*, actin-interacting protein 1 (Aip1) from yeast and *Xenopus* increased the ability of ADF/cofilin to sever filaments and increased the rate of filament disassembly [67,68]. Injection of *Xenopus* Aip1 caused the disassembly of filamentous actin in the contractile ring of cytokinesis [68]. In *Dictyostelium*, mutants lacking Aip1 are defective in endocytosis, motility, cytokinesis and growth, which involve actin assembly [69].

### A synthetic system of actin-based motility

The combination of purified Arp2/3, capping protein and ADF/cofilin was able to polymerize actin on the surface of *Listeria* and caused the bacteria to move [1<sup>\*\*</sup>]. This discovery represents the first description of pure proteins sufficient for motility based on actin polymerization. All three actin-binding proteins were necessary for *Listeria* motility; when the concentration of any one protein was zero, the speed of *Listeria* movement was zero [1<sup>\*\*</sup>]. As the concentration of each protein increased, the speed of *Listeria* movement increased to a maximum and then decreased.

*Listeria* is a good model for molecular analysis of actin polymerization. The movement of *Listeria* in cytoplasm is powered by actin polymerization, using host cell proteins. The actin polymerization and motility induced by *Listeria* are similar in many respects to the polymerization and motility that occur during extension of the leading edge of a cell. Furthermore, Arp2/3, capping protein and ADF/cofilin have all been implicated as important for actin polymerization and motility in cells.

Why is each protein necessary for motility? A proposed model for localized actin polymerization is shown in Figure 1. A localized stimulus activates Arp2/3 to nucleate actin polymerization with free barbed ends. For *Listeria*, the stimulus is the bacterial surface protein ActA. The free barbed ends polymerize for a time and are then capped by capping protein. The need for capping protein seems odd; it should inhibit polymerization. One explanation is that capping protein is bound mainly to the older barbed ends, which are located away from the activation zone, based simply on the fact that newly created barbed ends are free and some time is required for capping protein to associate with them. Therefore, in this situation, the presence of

capping protein restricts, or 'funnels', polymerization to the free barbed ends near the bacterium. ADF/cofilin is proposed to increase the rate of loss of subunits from pointed ends, increasing flux into the pool of free subunits, which then add to free barbed ends. This step requires removal of Arp2/3 from the pointed ends. Arp2/3 may dissociate spontaneously or in response to the change in filament structure upon ADF/cofilin binding.

The synthetic system may not provide a valid representation of how actin polymerization provides for motility *in vivo*. The system does reveal one way that this particular set of proteins can work together to provide motility, however, the cell is more complex, so these proteins may function differently and other proteins may play an important role. For example, in cell extracts, altering the activity of cofilin did not affect the speed of *Listeria* motility, only the length of the tail [70]. Perhaps in the cell extract, proteins other than ADF/cofilin, such as profilin and thymosin, provide for increased flux of subunits to free barbed ends [71]. In addition, *Rickettsia* in host cells displays actin-based motility with tails whose structure is different from that of *Listeria* [72,73]. The actin filaments in their tails are longer and less branched, resembling those of filopodia rather than lamellae; *Rickettsia* tails also do not contain Arp2/3 [73].

## Conclusions

Actin assembly involves the addition and loss of subunits at barbed and pointed ends of filaments. Actin-binding proteins have specific effects on these reactions. We are beginning to understand how these proteins, alone and in combination, can provide spatial and temporal control of actin polymerization *in vitro* and *in vivo*.

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