Cofilin takes the lead

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Summary

Cofilin has emerged as a key regulator of actin dynamics at the leading edge of motile cells. Through its actin-severing activity, it creates new actin barbed ends for polymerization and also depolymerizes old actin filaments. Its function is tightly regulated in the cell. Spatially, its activity is restricted by other actin-binding proteins, such as tropomyosin, which compete for accessibility of actin filament populations in different regions of the cell. At the molecular level, it is regulated by phosphorylation, pH and phosphatidylinositol (4,5)-bisphosphate binding downstream of signaling cascades. In addition, it also appears to be regulated by interactions with 14-3-3ζ and cyclase-associated protein. In vivo, cofilin acts synergistically with the Arp2/3 complex to amplify local actin polymerization responses upon cell stimulation, which gives it a central role in setting the direction of motility in crawling cells.

Key words: Stimulated protrusion model, Chemotaxis, Arp2/3 complex

Introduction

Directed cell movement is a fundamental process in many physiological contexts. During embryogenesis, it allows cells to move to the appropriate location. In adults, it is responsible for neutrophil- and macrophage-mediated chemotaxis and phagocytosis, as well as lymphocyte-mediated immune functions. The migration of fibroblasts and vascular endothelial cells is required for wound healing. Moreover, the migration of cells contributes to the underlying processes of life-threatening diseases, such as tumor invasion and metastasis.

Cell migration is a finely choreographed event consisting of several distinct steps. The cell initiates a protrusion at the front, which subsequently attaches to the substratum. This is followed by contraction of the cell body and tail detachment, resulting in movement in the direction of the protrusion. The cycle then repeats this process. The initial event in this cycle is a sensing of haptotactic and/or chemotactic signals by cell-surface receptors. These signals are communicated to the interior of the cell, where they activate a complex signaling pathway. The final outcome of this is polymerization of new actin at the leading edge, which generates protrusive force. Hence, the directionality of cell migration can be determined by these signals through their ability to set the location of the initial protrusion.

Extension of protrusions in response to migratory stimuli is coupled to actin polymerization. Actin filaments have barbed and pointed ends, the barbed end being the end at which actin monomers are incorporated with higher affinity during polymerization. For an actin monomer to incorporate at a barbed end, it has to be free, i.e. uncapped. For actin polymerization to occur adjacent to the plasma membrane there should, therefore, be an increase in the number of free barbed ends. Three different mechanisms to generate free barbed ends at the membrane exist: (1) uncapping of pre-existing barbed ends capped by capping protein or gelsolin-related proteins (Hartwig et al., 1995); (2) severing of filamentous (F)-actin by the actin-binding protein cofilin/ADF (Chan et al., 2000); and (3) de novo nucleation of filaments involving the Arp2/3 complex (Pollard et al., 2000) or formins (reviewed by Evangelista et al., 2003; Pruyne et al., 2002). Furthermore, the availability of local free monomeric G-actin might be an important factor regulating membrane protrusions. Cofilin/ADF, along with thymosin β-4 and profilin, may have an important role to play in modulating G-actin levels.

Cofilin/ADF (hereafter called cofilin) has emerged as one of the protein families playing an essential role in actin dynamics at the plasma membrane during cell protrusion (Bamburg, 1999; Condeelis, 2001; Ono, 2003; Pollard and Borisy, 2003). Cofilin is a small (19 kDa) ubiquitous protein that binds to both G- and F-actin; it has a higher affinity for ADP-bound subunits and enhances the rate of monomer dissociation from the pointed end of actin filaments (Bamburg, 1999; Carlier et al., 1997; Maciver et al., 1998). In addition, cofilin can also sever actin filaments and thus directly generate free actin barbed ends (Chan et al., 2000; Du and Frieden, 1998; Ichetovkin et al., 2000; Maciver et al., 1991). Both the depolymerization and severing activities of cofilin are presumably due to its ability to bind cooperatively to F-actin and cause a twist in the actin filament, which promotes the destabilization of actin-actin interactions and thus fragmentation of the filament (McGough et al., 1997).

Although the activation of cofilin is required for cell motility (Carlier et al., 1997; Condeelis, 2001), it was not clear until recently how the relative contributions of cofilin-mediated barbed-end formation and subsequent polymerization (Condeelis, 2001), and cofilin-mediated actin depolymerization (Carlier et al., 1997; Lappalainen and Drubin, 1997) are balanced during protrusion and cell motility. The relative contributions of these opposing functions of cofilin in vivo have been difficult to establish given comparisons of different cell types, in which the consequences of cofilin

activity depend on the initial availability of actin monomers and motility can be stimulated or constitutive. For example, Cramer has argued that, in migrating chick fibroblasts, cofilin-mediated depolymerization of actin filaments provides the actin monomers necessary for ongoing filament assembly (Cramer, 1999); by contrast, in neurons, the severing of actin filaments by cofilin to generate free barbed ends for actin polymerization is essential for growth cone motility (Endo et al., 2003). Here, we discuss recent findings that help to resolve how the polymerization and depolymerization activities of cofilin are balanced and have provided new insights into the function of cofilin in defining the direction of cell motility.

Synergy between Arp2/3 and cofilin

One of the major modulators of actin polymerization in cell protrusions is the Arp2/3 complex (Condeelis et al., 2001; Higgs and Pollard, 2001; Pollard and Borisy, 2003; Welch and Mullins, 2002). The Arp2/3 complex consists of seven polypeptides and is found at actin filament Y-branches in the submembrane dendritic array (Bailly et al., 1999; Svitkina and Borisy, 1999). Owing to its in vitro ability to generate new filament branches in a pre-existing actin filament network (Amann and Pollard, 2001; Blanchoin et al., 2000; Ichetovkin et al., 2002; Mullins et al., 1998; Pantaloni et al., 2000), it is believed to be a major contributor to barbed-end generation and cellular protrusion.

Several studies have shown that the Arp2/3 complex and cofilin are present together in dendritic arrays of actin filaments at the leading edge of motile cells (Bailly et al., 1999; Chan et al., 2000; Schafer et al., 1998; Svitkina and Borisy, 1999). More recently, analysis of growth-factor-stimulated live cells has shown that cofilin is recruited to the leading edge at a slightly faster rate than the Arp2/3 complex (DesMarais et al., 2004). Both the Arp2/3 complex and cofilin contribute to barbed-end generation at the leading edge, since function-blocking antibodies directed against either protein significantly decrease barbed-end generation and cell protrusion (Bailly et al., 2001; Chan et al., 2000).

Using a light microscopy assay in vitro that allows direct visualization of actin filaments, Ichetovkin et al. found that cofilin's severing activity and Arp2/3 complex's nucleation activity alone each cause a moderate increase in actin polymerization (Ichetovkin et al., 2002). However, together, they cause significantly more actin polymerization than the sum of their individual contributions (Ichetovkin et al., 2002). Experiments in live cells using function-blocking antibodies against either cofilin or the Arp2/3 complex mirror these findings. The experiments show that, when the contribution of either cofilin or the Arp2/3 complex is measured in the absence of the other, each alone contributes many fewer barbed ends than the number generated in control cells (DesMarais et al., 2004). This suggests synergy between the two pathways.

The primary finding of both studies is that the severing activity of cofilin can amplify the nucleation activity of the Arp2/3 complex. Amplification occurs because cofilin creates free barbed ends that nucleate the growth of new actin filaments, which are preferred sites for the binding of the Arp2/3 complex, compared with older, ADP-containing filaments. The availability of new actin filaments increases the nucleation activity of the Arp2/3 complex and biases its branching activity towards the barbed end of the mother filament. A similar argument has been made for the interaction between the Arp2/3 complex and gelsolin in platelets and fibroblasts, in which free actin filament barbed ends resulting from gelsolin severing are proposed to be necessary for the activity of the Arp2/3 complex (Falet et al., 2002).

The original steady-state protrusion model (Fig. 1), which has been proposed to explain in vitro biochemical data (Pollard et al., 2000), does not take the synergistic interaction between cofilin and the Arp2/3 complex into account. In this model, cofilin is proposed to function exclusively as an actin-recycling factor, depolymerizing filaments to yield G-actin. Depolymerization is required to sustain steady-state actin polymerization at the leading edge (Fig. 1A); it is tightly coupled to polymerization and is absolutely required for extension of the dendritic network. In this model, cofilin is proposed only to depolymerize F-actin, whereas the Arp2/3 complex near the cell membrane is responsible for dendritic nucleation. The model can explain the continuous uninterrupted movement of keratocytes and intracellular pathogens in cells, when G-actin levels limit polymerization. Consistent with the steady-state protrusion model is the observed depolymerization activity of cofilin in vitro and its localization to lamellipodia in keratocytes (Loisel et al., 1999; Svitkina and Borisy, 1999).

By contrast, the stimulated protrusion model (Fig. 1) considers the synergistic interaction between cofilin and the Arp2/3 complex. In addition, the amplification of Arp2/3-mediated dendritic nucleation by cofilin has several implications for models of cofilin function in vivo that are covered by this model. In many crawling cells, including fibroblasts and chemotactic carcinoma cells, movement is not continuous and is usually stimulated by some outside signal in the presence of an abundance of G-actin (>70 μM) (Edmonds et al., 1996). In crawling cells that exhibit a 'stop and start' mode of motion, actin depolymerization is not required for motility for many minutes after movement starts (Cramer, 1999). In such cells, cofilin is proposed to initiate the assembly of actin filaments and determine the timing and location of dendritic nucleation and its resulting protrusion. Results showing that cofilin is localized with barbed ends at the leading edge and not at the base of lamellipodia in stimulated carcinoma cells (Chan et al., 2000), and that cofilin is sufficient to generate barbed ends, actin polymerization and protrusion (Ghosh et al., 2004), support a direct role for cofilin severing in initiating and defining the direction of stimulated cell motility.

The two models are not mutually exclusive. A signal such as a chemoattractant could activate the stimulated protrusion mechanism, whereas sustained protrusive activity could involve the steady-state protrusion mechanism. The transition from a stimulated to a sustained protrusion mechanism could occur within the same cell if cell movement continues uninterrupted until G-actin concentrations become limiting to further polymerization, at which time depolymerization should be obligatorily coupled to further polymerization. The important difference between these two models is that, in the stimulated protrusion model, cofilin determines the sites of dendritic nucleation and, therefore, cell protrusion.

A photoactivatable analog of cofilin has been used to test the hypothesis that cofilin initiates actin polymerization and

determines sites of cell protrusion and directionality. Uncaging cofilin in vivo demonstrates that it generates free barbed ends, polymerizes actin, induces protrusion, and sets the direction of cell migration (Ghosh et al., 2004). Indeed, these studies indicate that cofilin serves as a dynamic component of the steering wheel of the cell. Recent analysis of the distribution of cofilin and phospho-cofilin in migrating fibroblasts supports a role for active cofilin at the leading edge and in establishing cell polarity (Dawe et al., 2003).

Regulation of cofilin activity

It is now apparent that cofilin can initiate protrusion and determine cell direction, and it is therefore important to understand how cofilin activity is regulated in cells with high spatial and temporal precision. Cofilin function appears to be tightly regulated in cells by: phosphorylation; protein-protein interactions involving tropomyosin, 14-3-3ζ and AIP1; and inositol phospholipids. In addition, the activity of cofilin is stimulated by an increase in pH (Bernstein et al., 2000; Hawkins et al., 1993; Yonezawa et al., 1985).

Cofilin phosphorylation

The severing and depolymerization activity of cofilin can be inhibited by phosphorylation on Ser3, which abolishes its actin-binding activity (Agnew et al., 1995; Moriyama et al., 1996). Four different kinases that appear to be downstream of the Rho-family GTPases phosphorylate cofilin: LIMK1, LIMK2, TESK1 and TESK2 (Arber et al., 1998; Dan et al., 2001; Rosok et al., 1999; Toshima et al., 2001; Yang et al., 1998). Phosphatases including type 1, type 2A (Ambach et al., 2000), type 2B (Meberg et al., 1998), type 2C (Zhan et al., 2003) and a novel cofilin phosphatase, slingshot (Niwa et al., 2002), have been implicated in reactivation of cofilin by dephosphorylation in a variety of cell types.

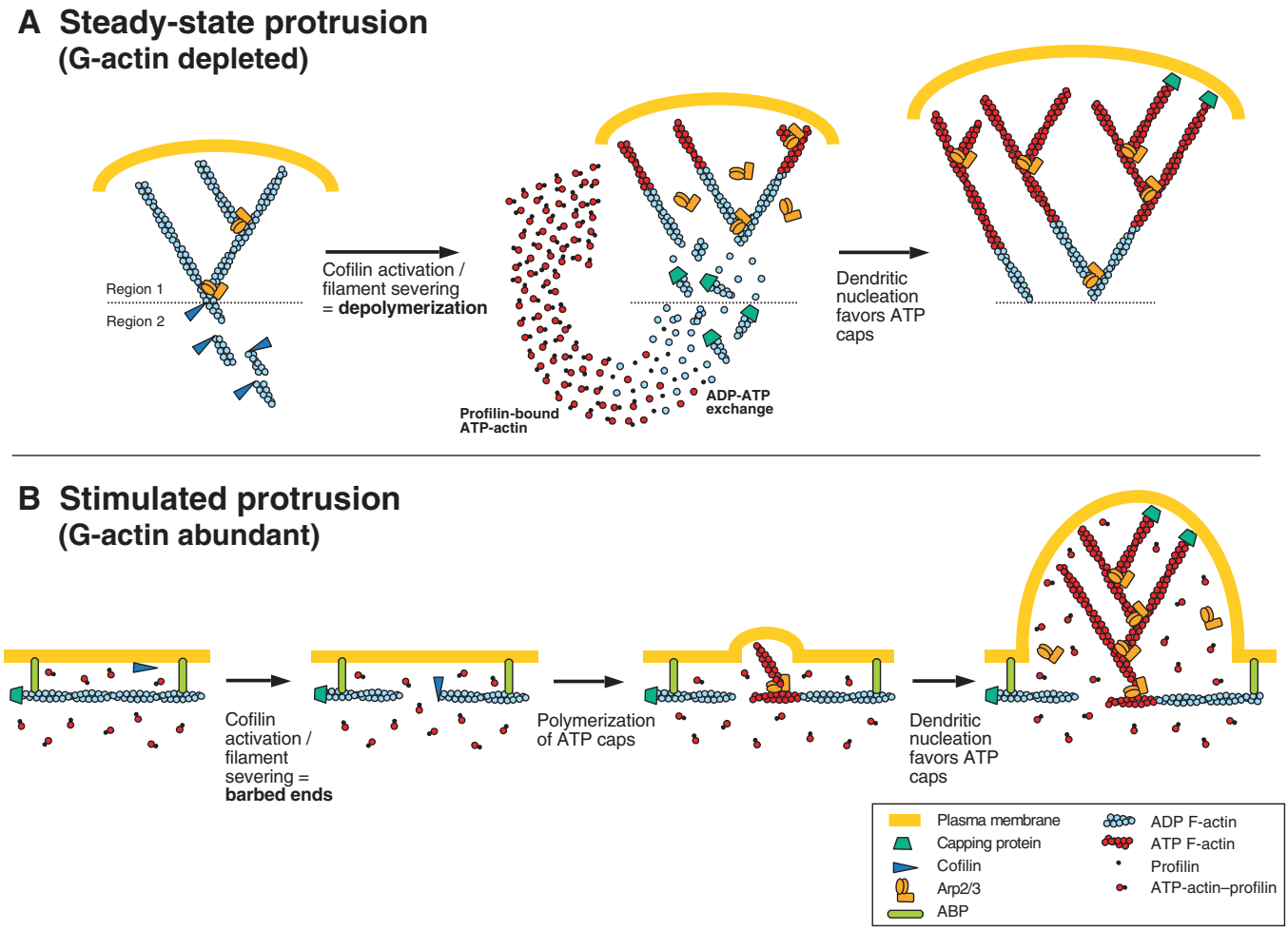

Fig. 1. The steady-state and stimulated protrusion models. (A) In the steady-state model [based on Pollard et al. (Pollard et al., 2000)], cofilin functions only as a G-actin-recycling factor, depolymerizing filaments to G-actin at the base of the lamellipodium to sustain steady-state actin polymerization at the leading edge when G-actin is limiting (e.g. in continuously moving cells such as keratocytes). Dendritic nucleation at the leading edge occurs from the Arp2/3 complex at the interface with the cell membrane. (B) The stimulated protrusion model applies to situations when motility is not continuous and G-actin is not limiting. In this case, initiation of movement involves the localized activation of cofilin at the leading edge. Severing of actin filaments in the quiescent cortical cytoskeleton by cofilin creates free barbed ends that define the site of activation of the Arp2/3 complex. Polymerization of actin occurs from a pool of pre-existing G-actin and is not tightly coupled to depolymerization.

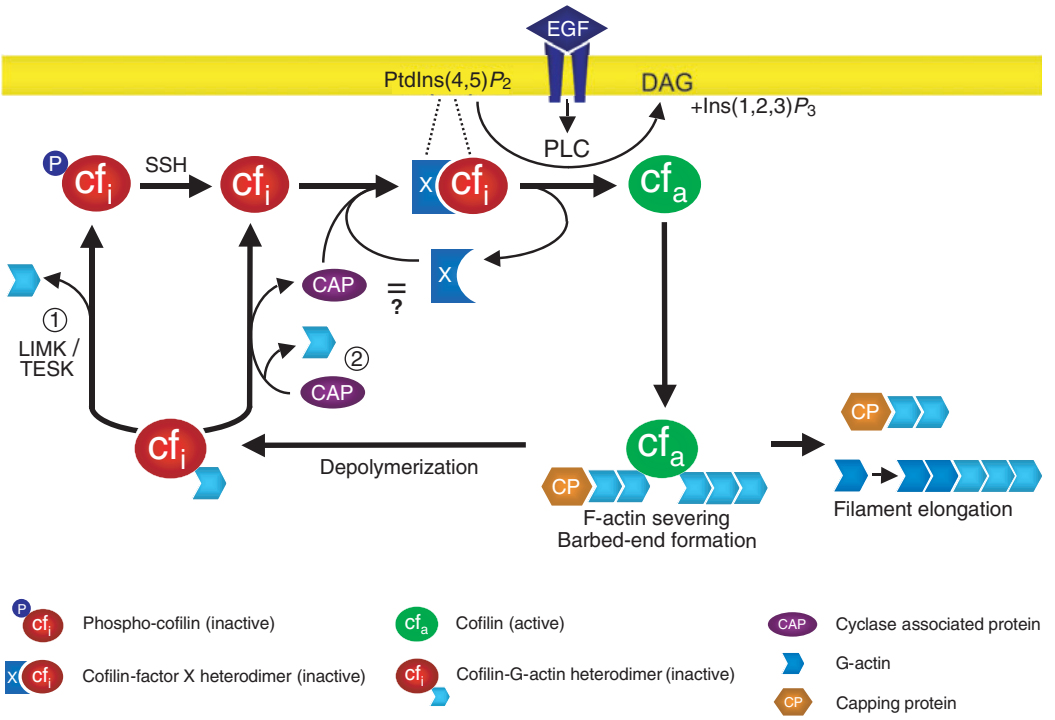

Fig. 2. A hypothetical model for the activity cycle of cofilin in crawling tissue cells. Some motile cells, such as neutrophils, regulate cofilin by dephosphoylation of an inactive phosphorylated pool upon chemotactic stimulation (not shown). By contrast, others, such as carcinoma cells, might maintain the majority of cofilin prior to stimulation in a dephosphorylated yet inactive state (cf_i) generated through interaction of cofilin with PtdIns(4,5)P_2 and/or formation of cofilin–G-actin heterodimers. Following EGF stimulation, a PLC-dependent step releases activated cofilin (cf_a), which then associates with F-actin to promote F-actin severing. This leads both to polymerization and depolymerization, the balance being determined by the relative availability of G-actin. Cofilin is rescued from the cofilin–G-actin heterodimer by two mechanisms. (1) Phosphorylation by LIM kinase (LIMK) or TES kinase (TESK) turns off the actin-binding activity of cf, releasing G-actin and phospho-cofilin. Cofilin phosphatases such as PP1, PP2A or SSH (slingshot) can then replenish the pool of dephosphorylated cofilin. (2) CAP can bind to the cofilin–G-actin heterodimer and release free cofilin and G-actin. The freed cofilin can bind to PtdIns(4,5)P_2 to form an inhibitory complex that is released locally by EGF-stimulated receptors to begin the activity cycle again. Cofilin may bind directly to PtdIns(4,5)P_2 or through another protein (X). CAP is a candidate for X since it regulates cofilin location in vivo. Localized activation of cofilin by PtdIns(4,5)P_2 hydrolysis causes local actin polymerization and protrusion, and sets the direction of movement.

The importance of regulation of cofilin by phosphorylation seems to vary by cell type. In some resting cells, cofilin is mostly phosphorylated (Moriyama et al., 1996). Stimulation of motility by a variety of agents induces dephosphorylation and activation of cofilin (Kanamori et al., 1995; Okada et al., 1996). In carcinoma cells, at least half of cofilin is in the dephosphorylated state, and yet cofilin is inactive (Chan et al., 2000; Zebda et al., 2000). Furthermore, cofilin is rapidly phosphorylated upon epidermal growth factor (EGF) stimulation, which indicates a more complex regulatory mechanism than simply the dephosphorylation of cofilin (Mouneimne et al., 2004). This is discussed below and shown in Fig. 2. Hence, the dephosphorylation of cofilin by activation of cofilin phosphatases following *N*-formyl-methionyl-leucyl-phenylalanine (fMLF) stimulation, as observed in neutrophils (Okada et al., 1996; Suzuki et al., 1995), is unlikely to be the main regulatory pathway to cofilin activation in cultured cells.

The protein 14-3-3ζ binds to phosphorylated cofilin and prevents its dephosphorylation (Gohla and Bokoch, 2002) and may thus be involved in regulating the phosphorylation state of cofilin. AIP1 also binds to cofilin (Aizawa et al., 1999; Okada et al., 1999; Ono, 2003; Rodal et al., 1999) and may stimulate the depolymerization activity of cofilin (reviewed by Ono, 2003).

Tropomyosin

The binding of tropomyosin to actin filaments prevents them from being depolymerized (Bernstein and Bamburg, 1982) or severed (DesMarais et al., 2002) by cofilin. Tropomyosin also prevents the Arp2/3 complex from binding to filaments to initiate branches (Blanchoin et al., 2001). Thus, tropomyosin may be able to restrict spatially the activities of cofilin and the Arp2/3 complex in vivo to certain populations of actin filaments in certain compartments of the cell (reviewed by Cooper, 2002; Ono, 2003). In the muscle cells of *Caenorhabditis elegans*, tropomyosin stabilizes actin filaments by protecting them from cofilin-mediated filament disassembly (Ono and Ono, 2002). In carcinoma cells, the dynamic nucleation zone at the leading edge of the lamellipod is enriched in cofilin and the Arp2/3 complex, but tropomyosin is depleted from this region of the cell and is present mainly on actin filaments in the cell body and on stress fibers (DesMarais et al., 2002). This allows the establishment of functionally distinct actin compartments in the cell, with rapid

generation of actin barbed ends in the cofilin- and Arp2/3-complex-rich leading edge compartment, and very little barbed-end formation in the tropomyosin-rich cell body.

The regulation of actin dynamics by tropomyosin and cofilin might differ between cell types. Whereas in carcinoma cells and *C. elegans* muscle cells, tropomyosin and cofilin functions are antagonistic, in neuroblastoma cells that overexpress the tropomyosin isoform TM5, the neuron-specific tropomyosin isoform TmBr3, which binds to F-actin weakly (Hammell and Hitchcock-DeGregori, 1996), associates with cofilin-bound actin filaments (Bryce et al., 2003). This suggests that some tropomyosin isoforms, such as TM5, can specifically regulate the incorporation of other tropomyosin isoforms, such as TmBr3, into actin filaments – for example, by selective dimerization. TmBr3 appears to be an example of a tropomyosin isoform that does not compete for actin binding with cofilin and thus may not limit cofilin activity.

Cyclase-associated protein

Cofilin binds to G-actin with micromolar affinity and the heterodimer is inactive in both severing and depolymerization (Bamburg, 1999). Recently, it was demonstrated that cyclase-associated protein (CAP) stimulates the release of cofilin from the cofilin–G-actin heterodimer (Moriyama and Yahara, 2002) and aids in the depolymerization of F-actin from the pointed end (Balcer et al., 2003; Moriyama and Yahara, 2002). CAP also seems to be able to support barbed-end elongation by enhancing the exchange of ADP- to ATP-actin (Moriyama and Yahara, 2002). This in turn increases the rate of actin polymerization and depolymerization in vivo and determines the location of cofilin in crawling cells (Bertling et al., 2004). Depletion of CAP expression leads to defects in filament turnover and cell polarity consistent with a role for cofilin in determining cell direction (Bertling et al., 2004). The abundance of CAP, the fact that CAPs are highly conserved actin-binding proteins present in all eukaryotes, and the involvement of CAP in releasing cofilin from its inhibitory complex with G-actin make CAP a candidate for regulating the dephosphorylated population of cofilin in vivo in many cell types (Fig. 2).

Phosphatidylinositol (4,5)-bisphosphate binding/phospholipase Cγ

Another mechanism of regulating cofilin function is through its binding to phosphatidylinositol (4,5)-bisphosphate [PtdIns(4,5)P_2], which inhibits the actin-binding activity of cofilin (Ojala et al., 2001; Yonezawa et al., 1991; Yonezawa et al., 1990). This mechanism is particularly suitable for the spatial regulation of cofilin activity at the leading edge.

Phospholipase C (PLC) is an important regulator of cell crawling (Kassis et al., 1999). It is thought to remodel the actin cytoskeleton through PtdIns(4,5)P_2 hydrolysis, leading to activation of cofilin (Yonezawa et al., 1991; Yonezawa et al., 1990), gelsolin (Allen, 2003; Chou et al., 2002; Sun et al., 1999; Yonezawa et al., 1990) and profilin (Goldschmidt-Clermont et al., 1992; Goldschmidt-Clermont et al., 1991). Cofilin, profilin and gelsolin are postulated to bind to PtdIns(4,5)P_2 in an inhibitory complex in resting cells; when released upon PtdIns(4,5)P_2 hydrolysis, they locally remodel the actin cytoskeleton. Activated PLCγ1 can hydrolyze PtdIns(4,5)P_2 that is bound to profilin, leading to its release and activation (Goldschmidt-Clermont et al., 1991). A similar mechanism might release cofilin from PtdIns(4,5)P_2 upon EGF stimulation of carcinoma cells. Profilin can support subsequent actin polymerization by facilitating nucleotide exchange and biasing actin monomers towards barbed-end polymerization (dos Remedios et al., 2003). Gelsolin and cofilin affect actin polymerization by severing.

Gelsolin severs actin filaments in a Ca^{2+}-dependent manner and finishes by capping the barbed ends until membrane PtdIns(4,5)P_2 levels are restored sufficiently to cause uncapping (Sun et al., 1999). This is a relatively slow process (with a half life of 15 minutes after growth factor stimulation) (Allen, 2003) and is inconsistent with gelsolin contributing to stimulation of protrusion, which occurs rapidly after receptor activation (Mouneimne, 2004). However, in some cell types, such as platelets and fibroblasts, gelsolin appears to contribute to the generation of barbed ends since gelsolin-null platelets have 45% fewer barbed ends after stimulation (Falet et al., 2002). Actin assembly is also reduced in gelsolin-null platelets and fibroblasts (Azuma et al., 1998; Falet et al., 2000). In other motile cells, such as carcinoma cells, a better case can be made for the involvement of cofilin in the rapid actin polymerization transients observed after stimulation, because of its fast response and the evidence discussed above implicating cofilin and the Arp2/3 complex as major contributors to production of barbed ends in these cells. Careful analysis of the timing of the contributions of cofilin and gelsolin to barbed-end production is needed to resolve their relative effects on stimulated and steady-state actin polymerization.

In several cell types, stimulated cell motility requires a biphasic actin polymerization transient (Chan et al., 1998; Chen et al., 2003; Cox et al., 1992; Eddy et al., 1997; Funamoto et al., 2002; Hall et al., 1989; Iijima and Devreotes, 2002). Recent work (Mouneimne et al., 2004) has demonstrated that the early transient of barbed ends coincides with a peak of PLC activity and that both this PLC activity and cofilin activity are required for the early but not the late transient of barbed ends. More specifically, PLC activity is required for the protrusion of the cell towards a source of EGF, which indicates that PLC determines the directionality of the protrusion. Furthermore, inhibitor studies show that the activation of cofilin during the early transient requires PLC activity, suggesting that PLC regulates the early barbed-end transient through cofilin. By contrast, inhibition of phosphoinositide 3-kinase activity suppresses the late but not the early transient, which is consistent with its involvement in the generation of protrusive force but not in the sensing of chemotactic signals (Mouneimne et al., 2004).

The physiological equivalent of local uncaging of cofilin (Ghosh et al., 2004) might therefore be the local hydrolysis of PtdIns(4,5)P_2 by activated PLCγ, causing the release of active cofilin locally. In this scenario, the localized formation of new barbed ends by cofilin would lead to localized dendritic nucleation by the Arp2/3 complex and localized protrusion. This would set the direction of cell movement during chemotaxis.

Conclusions

Recent evidence indicates that cofilin controls sites of actin polymerization and protrusion. This places cofilin in the unexpected role of a decision maker during chemotaxis that helps to determine the direction of movement in response to a chemoattractant. Cofilin might also influence overall cell polarity by the same molecular mechanism, which involves its synergy with the Arp2/3 complex to stimulate dendritic nucleation. These considerations lead to two models for cofilin function in vivo: the stimulated and steady-state protrusion models. These models can explain how cells can change direction quickly in response to exogenous signals and switch to continuous, persistent motion in a single direction, using the same underlying machinery for generating protrusive force. The challenge for the future is to understand how cofilin is regulated with high spatial and temporal precision as the steering wheel of the cell.

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