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CELL SCIENCE AT A GLANCE

Regulators of actin filament barbed ends at a glance

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ABSTRACT

Cells respond to external stimuli by rapidly remodeling their actin cytoskeleton. At the heart of this function lies the intricately controlled regulation of individual filaments. The barbed end of an actin filament is the hotspot for the majority of the biochemical reactions that control filament assembly. Assays performed in bulk solution and with single filaments have enabled characterization of a plethora of barbed-end-regulating proteins. Interestingly, many of these regulators work in tandem with other proteins, which increase or decrease their affinity for the barbed end in a spatially and temporally controlled manner, often through simultaneous binding of two regulators at the barbed ends, in addition to standard mutually exclusive binding schemes. In this Cell Science at a Glance and the accompanying poster, we discuss key barbed-end-interacting proteins and the kinetic mechanisms by which they regulate actin filament assembly. We take F-actin capping protein, gelsolin, profilin and barbed-end-tracking polymerases, including formins and WH2-domain-containing proteins, as examples, and illustrate how their activity and competition for the barbed end regulate filament dynamics.

KEY WORDS: Actin assembly, Capping protein, Filament barbed end, Formin, Motility, Profilin

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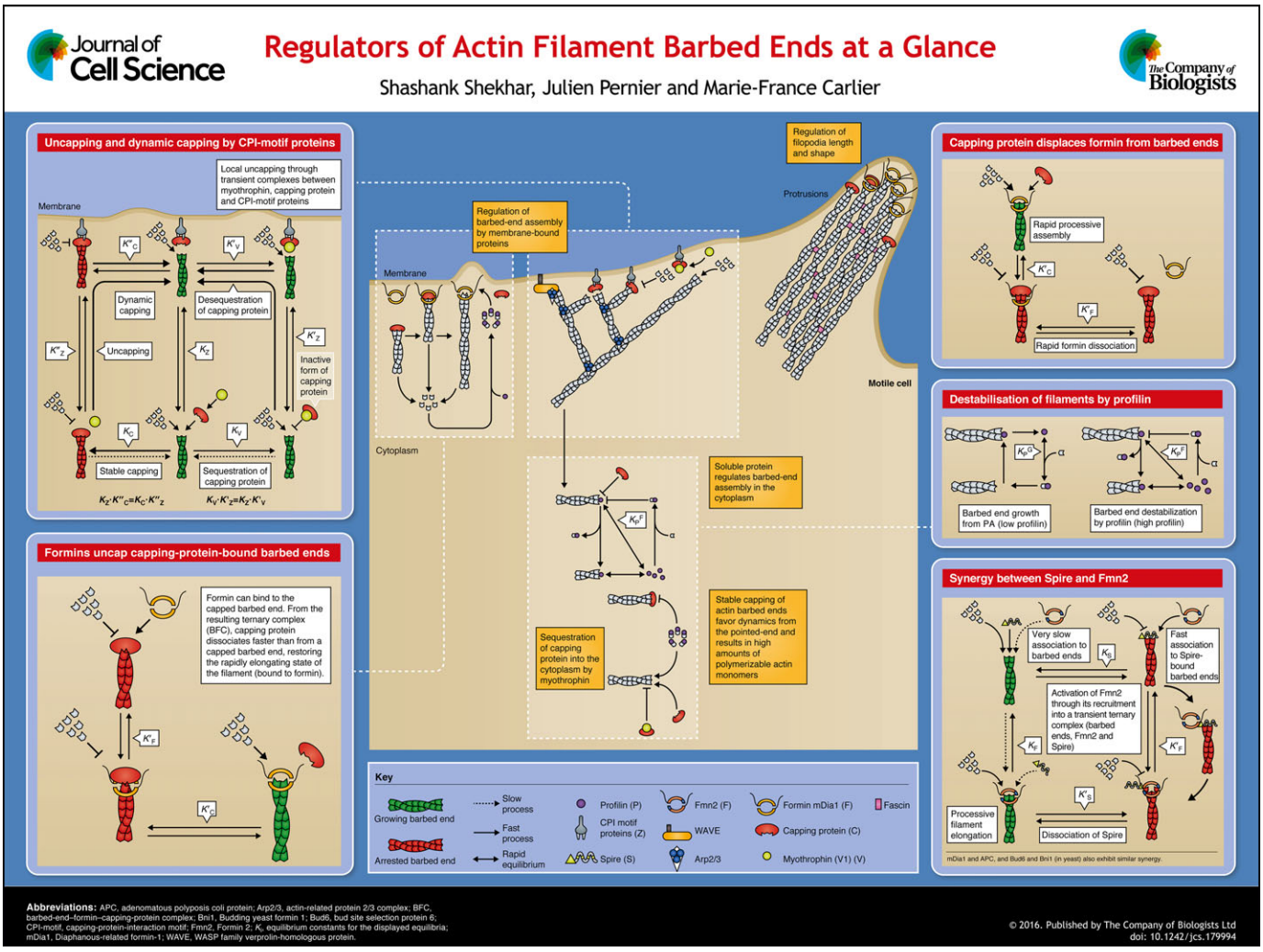
Introduction

Remodeling of the actin cytoskeleton provides the driving force for shape changes that cells use to migrate, feed and divide (Bugyi and Carlier, 2010; Carlier et al., 2015). Polarized nucleation and growth of actin filaments is controlled in rate, time and space by a large number of regulatory proteins. Actin filaments are polar structures



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whose two ends are referred to as the 'barbed end' and the 'pointed end'. When actin filaments coexist with actin monomers at steady state in the presence of ATP, monomer addition occurs predominantly at the barbed end, whereas disassembly mainly takes place at the pointed end. In the cellular context, various filament arrays are in constant turnover through dynamic, vectorial monomer–polymer exchanges in an ATP-rich medium. As a result, the pool of monomers is constantly replenished and remains stationary (i.e. non-finite and non-exhaustible). Under these conditions, the growth of filament barbed ends is fed steadily, and there is no competition for actin monomers between the various filament arrays. The cellular context thus differs from a putative *in vitro* situation, in which a finite and exhaustible pool of globular (i.e. monomeric) actin (G-actin) subunits is made available for filament assembly by several different nucleators at time zero.

Although the individual functions of barbed-end-binding proteins are well known, it is not clear how they work together in various cellular processes to maintain harmonious cellular activities. The binding sites of these proteins overlap to various extents on the two terminal actin subunits that constitute the filament barbed ends. This raises the question of how the competition for barbed-end-binding is controlled in individual actin-based processes, and beyond, at the cell level.

Newly discovered mechanisms expand the repertoire of the regulation of actin dynamics. In addition to the simple mechanism of a mutually exclusive binding of two proteins to barbed ends, two different barbed-end-binding proteins can also bind simultaneously to barbed ends with mutually reduced affinities and enhanced dissociation rates (Bombardier et al., 2015; Shekhar and Carlier, 2016; Shekhar et al., 2015). Thus, fast changes in reactivity of the barbed ends, from being blocked to full speed growth are elicited. These mechanisms, surveyed in this poster article, have been established using bulk solution, as well as single-filament and single-molecule kinetics. They shine light on the necessary functional link between the dynamics of cytosolic filamentous actin (F-actin), G-actin and membrane-attached actin filaments at the intracellular steady-state level. In addition, they point to the potential importance of the structural plasticity of filament barbed ends.

Regulation of barbed end capping by various capping proteins

Cappers are a class of proteins that tightly bind barbed ends to block further addition of monomers. This reaction also causes abortion of filament nucleation. A wide array of cappers has been reported *in vivo*. First, the class of Ca^{2+}-dependent capping proteins of the gelsolin superfamily, including gelsolin, adseverin, villin, advillin, supervillin, flightless I homolog and CapG in mammalian cells, and brevin, severin and fragmin in lower eukaryotes (Nag et al., 2013). They generally comprise six so-called gelsolin-type domains (CapG harbors the first three domains only) and sever and cap filaments with very high affinity. Although gelsolin is present in micromolar amounts in cells, it is difficult to evaluate its concentration in the Ca^{2+}-activated state since the affinity of Ca^{2+} ions for two types of Ca^{2+}-binding sites on gelsolin greatly depends both on pH and on actin binding to gelsolin. By severing the filaments, these proteins create a large number of very tightly capped short filaments that depolymerize at their pointed ends, within a physiological context. Phosphatidylinositol 4,5-bisphosphate (PIP_2) is the only known uncapper of gelsolin and might facilitate barbed-end growth against the membrane (Janmey and Stossel, 1987).

Capping protein (an heterodimer of an α-subunit and a β-subunit), the homolog of the striated muscle protein CapZ, is the major barbed-end capper in non-muscle cells from yeast to higher eukaryotes and is abundant (1 to 2 µM) (Edwards et al., 2014). It binds to the two terminal actin subunits with high affinity (K_d=0.1 nM; see Box 1 and poster). Consistently, it dissociates very slowly ($t_{1/2}$=25 min) from barbed ends (Schafer et al., 1996). If all intracellular capping protein was active, barbed ends would be permanently capped in cells, inhibiting all actin-based movements. Therefore, the activity of capping protein needs to be regulated *in vivo*. Capping-protein-interacting proteins achieve this in two ways (see Edwards et al., 2014 for a review). First, some proteins such as myotrophin (also known as protein V1), which is also present at micromolar amounts, sequester free capping protein by binding to its actin-binding site with high affinity (K_d=20 nM, denoted K_V, Box 1; Bhattacharya et al., 2006; Zwolak et al., 2010). Thus, the concentration of free capping protein available for barbed-end capping is buffered to 5–50 nM by myotrophin (see poster). Second, proteins of the 'capping protein interaction' (CPI)-motif-containing family [including e.g. CARMIL (capping-protein, Arp2/3 and myosin-I linker; also known as LRRC16A), CapZIP (CapZ-interacting protein, also known as RCSD1), FAM21 and CIN85 (c-Cbl-interacting protein of 85 kDa, also known as SH3KBP1 and CD2AP) and Duboraya (a zebrafish homolog of CapZIP);

Box 1. Thermodynamic parameters for the regulation of filament barbed-end dynamics as described on the poster.

Parameters are shown for barbed-end capping by capping protein and its sequestration by myotrophin (V1), for uncapping and dynamic capping by CPI-motif-containing proteins and by formins, and for regulation of barbed-end dynamics by profilin. The equilibrium dissociation constants are denoted K_i, and are as shown on the poster. The total concentrations of capping protein (C_0) and myotrophin (V_0) are taken as 1 µM and 3 µM, respectively (Fujiwara et al., 2014). The concentration of the myotrophin–capping-protein complex (VC) is calculated as $\{C_0+V_0+K_V\pm([C_0+V_0+K_V]^2-4\ V_0{\bullet}C_0)^{1/2}\}/2$, leading to a free capping protein concentration of 10 nM. Detailed balance imposes $K_V{\bullet}K'_Z=K_Z{\bullet}K'_V$, $K_Z{\bullet}K''_C=K_C{\bullet}K''_Z$, and $K_C{\bullet}K'_F=K_F{\bullet}K'_C$. B, barbed end; F, formin; Z, CPI-motif protein; BXY, complex of barbed end with X and Y proteins (X and Y can be C, F or Z)

Barbed-end regulation in the cytoplasm

Capping protein sequestration by myotrophin (K_V=V•C/VC)=20 nM (Fujiwara et al., 2014)

Barbed-end capping by capping protein (K_C=B•C/BC)=0.1 nM (Fujiwara et al., 2014)

Profilin binding to G-actin (K_P^G)=0.1 µM (Kinosian et al., 2000)

Profilin binding to barbed ends (K_P^F)=25 µM (Jegou et al., 2011)

Barbed-end regulation at membranes

Binding of CPI-motif-containing proteins to capping protein (K_Z=C•Z/CZ) =1 nM (Fujiwara et al., 2014)

Binding of myotrophin to a capping-protein–CPI-motif-containing complex (K'_V/V•CZ/VCZ)=1.3 µM (Fujiwara et al., 2014)

Binding of myotrophin–capping-protein to CPI-motif-containing protein (K'_Z=VC•Z/VCZ)=618 nM (Fujiwara et al., 2014)

Binding of the capping protein and CPI-motif-containing complex to barbed ends (K''_C=B•CZ/BCZ)=38 nM (Fujiwara et al., 2010)

Binding of a barbed-end–capping-protein complex to CPI-motif-containing proteins (K''_Z=BC•Z/BCZ)=211 nM (Fujiwara et al., 2010)

Capping protein and formin cross-regulation

Capping protein binding to formin-bound barbed ends (K'_C=BF•C/BFC)≈20 nM (Shekhar et al., 2015)

Formin binding to barbed ends (K_F=B•F/BF)=0.0034 nM (Shekhar et al., 2015)

Formin binding to capped barbed ends (K'_F=BC•F/BCF)=4 nM (Shekhar et al., 2015)

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collectively indicated by the subscript 'Z' in the equilibrium constants on the poster] lower the affinity of capping protein for barbed ends through an allosteric mechanism (Takeda et al., 2010). They bind to both free capping protein and barbed-end-bound capping protein through CPI motifs located at a non-actin-binding site, thus reducing the affinity of capping protein for actin; this results in an enhanced rate of dissociation of capping protein from barbed ends (uncapping). These proteins are therefore sometimes also called 'uncappers'. Conversely, a CPI-containing protein displays an identically reduced affinity for capping protein in the ternary complex formed by the barbed end, capping protein and a CPI (Fujiwara et al., 2010) (see poster). Similarly, CPI-containing proteins can bind to the capping-protein–myotrophin complex, which promotes the fast release of myotrophin (Fujiwara et al., 2014; Takeda et al., 2010). In summary, CPI-containing proteins locally regulate the dynamics of both capping protein sequestration and barbed-end capping, through ternary complexes.

Interestingly, proteins of the CPI family act in a site-specific manner, mainly at locations where Arp2/3-branched filaments are assembled (Fujiwara et al., 2014). For instance, FAM21 is a subunit of the WASH complex and CIN85 is a ligand of N-WASP (also known as WASL), which acts in endocytosis, whereas CARMIL is an Arp2/3–myosin-I linker and localizes at protruding cell edges. Thus, depending on the concentration of capping protein and the local density of CPI-containing proteins, either uncapping or capping with a dynamic rapid equilibrium takes place locally 'on-demand' at sites of protrusion (see poster). In contrast, in the cytoplasm, stable capping due to slow dissociation of capping protein from barbed ends prevents any unproductive polymerization of monomeric actin and the associated futile ATP consumption. The physiological function of capping protein actually requires its ability to bind both actin and CPI-containing proteins (Edwards et al., 2015).

Uncapping can also occur through the transient formation of a ternary complex, in which another barbed-end regulator associates with the capping-protein-bound terminal actin subunits at the barbed end (see poster). In this transient ternary complex, each of the two ligands displays a lower affinity for the barbed end than when it binds individually. The WH2 domain present in VopF, an effector of the pathogen *Vibrio cholerae*, and the FH2 domain of formins, have been identified as such 'uncappers' (Pernier et al., 2013; Shekhar et al., 2015). VopF, owing to its structural organization with multimerized WH2 repeats, has been proposed as a functional homolog model for Ena or VASP family proteins (Pernier et al., 2013). This family of proteins, however, is so far acknowledged only as 'anti-capping' proteins (Edwards et al., 2014). Although no uncapping activity has been formally established for VASP, only uncapping can account for available data (see figure 2D of Barzik et al., 2005), which show that capping-protein-capped filaments (from which capping protein dissociates with a half-time of 25 min) immediately start to rapidly depolymerize upon dilution in the presence of VASP. Hence, uncapping appears as a general property of multimerized WH2 repeats, and might be also involved in the regulation of filopodia length. Following dissociation of capping protein from the ternary complex, formin or VASP remains bound to and tracks growing barbed ends. Both the WH2 and FH2 domains possess a major actin-binding motif that consists of a short amphipathic α-helix that inserts into a hydrophobic pocket at the barbed face of actin in the shear zone between subdomains 1 and 3 (see Carlier et al., 2015 for a review). One of the actin-binding elements of capping protein, the β-tentacle, might thus compete with the 'knob' of FH2 and the N-terminal α-helix of WH2 domains for binding to actin, whereas other regions in these proteins maintain their interaction with other sites on actin. This partial overlap enables the formation of ternary complexes between the terminal actin subunits at the barbed end and two regulatory proteins.

The dynamics of the ternary complex formed by two proteins with the actin subunits at the barbed end depends on the strength of the different interfaces of each protein with actin. These interfaces include the aforementioned amphipathic α-helices that target the hydrophobic cleft at the barbed face of actin, as well as other actin-binding elements. To be specific, the ternary complex formed by VopF and capping protein at barbed ends appears to be in rapid equilibrium with the free ligands (Pernier et al., 2013). In contrast, the ternary complex between formin, capping protein and the barbed ends has a longer life-time and dissociates into formin-bound and capping-protein-bound barbed ends in proportions that are defined by the relative rate constants for the dissociation of each protein from the ternary complex (Shekhar et al., 2015).

Note that the uncapping reaction has been analyzed so far only at a simplistic level. A change in affinity owing to hydrolysis of ATP on terminal subunits would introduce subtleties that are not expressed in the isoenergetic square model that describes binding of capping protein and formin to barbed ends (Shekhar et al., 2015).

Finally, in the transient ternary complex, the structural organization of the two terminal actin subunits that compose the barbed end might differ from their conformation in the free (unliganded) state or the single-ligand-bound state at the barbed end. These putative structural changes might potentially facilitate (or reduce) binding of other ligands.

Regulation of processive filament assembly at the barbed end by formins

Downregulation of formin activity

Like capping protein, most formins bind to barbed ends with high affinity (K_d<<1 nM) through their FH2 domains and display a very slow dissociation rate (10^{-4} s^{-1} range) (Kovar et al., 2006; Romero et al., 2004). Thus, a single formin molecule engaged with the barbed end catalyzes thousands of consecutive cycles of endwise association of profilin–actin without detaching from the filament (see poster). This behavior is referred to as 'processivity'. *In vitro*, this long dwell time generates filaments that are several tens of microns long, much longer than the intracellular lengths of formin-assembled filaments (e.g. in filopodia or in stress fibers). The length of formin-assembled filaments can be negatively controlled in two ways, either by lowering the rate of processive assembly, or by enhancing formin dissociation from barbed ends. Both mechanisms exist *in vivo*.

As an example of the former possibility, profilin, which is required for the fast, formin-mediated processive assembly, slows down the formin-based elongation at high concentrations (see also section on profilin below). Thus, the rates of processive assembly and of formin-based propulsion display a bell-shaped dependence on the profilin concentration (Kovar et al., 2006; Pernier et al., 2016). Additionally, Smy1 in yeast slows down the processive assembly that is mediated by the yeast formin Bnr1 through an unknown mechanism (Chesarone-Cataldo et al., 2011). Here, the residence time of the formin is reduced by enhancing its dissociation from the barbed end. In yeast, dissociation of formin from barbed ends is enhanced by Bud14, which binds and inhibits the FH2 domain of formin in cooperation with Kelch proteins (Chesarone et al., 2009; Gould et al., 2014).

Dissociation of formin from barbed ends is also enhanced by capping protein as described above through the formation of a transient ternary barbed-end–formin–capping-protein complex

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(hereafter 'BFC' complex) in which the affinity of both formin and capping protein for the barbed end is lowered (Bombardier et al., 2015; Shekhar et al., 2015) (see poster). Filament growth at the barbed end is halted in the BFC state as it is when the capping protein binds the barbed end, consistent with capping-protein-mediated inhibition of both free barbed-end and processive barbed-end growth. Upon exposure to profilin–actin, either capping protein or the formin (mDia1) dissociates from the ternary complex (BFC) at rates that are the same order of magnitude, resulting in 25% formin-bound barbed ends (which are rapidly growing) and 75% capping-protein-bound barbed ends (which are paused). However the distribution between barbed ends bound to formin and those bound to capping protein would favor the a capping-protein-bound state even more if a more weakly binding formin or a more tightly binding capping protein was bound in the BFC complex. Conversely, the distribution might be in favor of a formin-bound barbed end if a more weakly bound capper [such as Eps8 or Spire (for which there are two isoforms in mammals, Spire1 and Spire2)] was bound.

When formins are anchored, mimicking the physiological context at filopodia or lamellipodia tips, binding of capping protein to barbed ends results in a transient arrest of growth and rapid detachment of the capping-protein-capped filaments from formin (Shekhar et al., 2015). These *in vitro* results provide the first reported evidence of a direct regulation of formins by capping protein. They also support recent *in vivo* observations that the presence of capping protein in filopodia is responsible for their tapered shape, suggesting that capping protein regulates formin-bound barbed ends in filopodia (Sinnar et al., 2014).

Upregulation of formin activity

Some formins are inherently poor nucleators and require an activator for their function (see poster). A number of formin activators have recently been identified, but the detailed mechanisms by which they enhance formin activity are generally not clear. In yeast, Bud6 was first described as a nucleation cofactor for the formins Bni1 and Bnr1 (Graziano et al., 2013). However, recent work reveals that, in addition to interacting with barbed-end-bound protein Bni1, Bud6 also binds the barbed face of actin, using an amphipathic α-helix similar to the one present in WH2-domain-containing proteins (Park et al., 2015). Under conditions of processive elongation, the actin-binding site for Bud6 is exposed only at Bni1-bound barbed ends, as G-actin is essentially bound to profilin. Similarly, the adenomatous polyposis coli (APC) protein, which by itself binds to actin and stimulates filament assembly, associates and synergizes with the formin mDia1; this, in turn, further stimulates actin assembly, potentially using a similar mechanism (Breitsprecher et al., 2012; Okada et al., 2010).

In *Drosophila* oogenesis and mouse meiosis, formin 2 (Fmn2; Cappuccino in *Drosophila*) requires Spire to elicit the assembly of a dense cytoplasmic actin meshwork that is required for axis patterning in *Drosophila* and for asymmetric spindle positioning in the mouse oocyte (Dahlgaard et al., 2007; Pfender et al., 2011). *In vitro* approaches using bulk solution and single-filament kinetics have shown that Fmn2 by itself nucleates poorly from profilin–actin and has a very low affinity for the barbed ends (Montaville et al., 2014). Spire, which by itself caps barbed ends through its WH2 domains (Bosch et al., 2007), interacts directly with the C-terminal extension of the FH2 domain of Fmn2, termed FSI (for formin-Spire-interaction) through its N-terminal KIND domain (Vizcarra et al., 2011; Zeth et al., 2011). The association of Fmn2 to Spire-bound barbed ends is followed by fast processive assembly, during which Spire is displaced from the barbed end (Montaville et al., 2014). Recent studies indicate that the FSI region also binds to barbed ends in a similar manner to a WH2 domain and is required for the processive activity of Fmn2 (Montaville et al., 2016; Vizcarra et al., 2014). Accordingly, deletion of the FSI region converts Fmn2 into a capping protein that is bound to barbed ends in the 'closed' conformation. The FSI domain might increase the fraction of time spent by Fmn2 in the 'open' conformation by outcompeting the knob region of FH2 (Montaville et al., 2016).

The identification of structural elements involved in competition at barbed ends sheds light into the regulatory mechanisms of individual formins, which contain several actin-binding domains. Specifically, formins such as INF2, FMNL2 and FMNL3 (Chhabra and Higgs, 2006; Heimsath and Higgs, 2012) contain WH2 or WH2-like domains in their C-terminal tail, in addition to their FH2 domains, which bind barbed ends and accelerate processive polymerization. The aforementioned functional similarity between the FSI of formin 2 and a WH2 domain suggests that the C-terminal extensions of various formins might play similar roles in assisting processive elongation.

Regulation of formins by tensile force

Formins often direct processive filament assembly from membrane-bound sites, such as at the bud neck of *Saccharomyces cerevisiae*, at the tip of filopodia, at spots in the contractile cytokinetic ring or at specific sites in podosomes. How tension affects the activity of formins has been addressed only *in vitro* so far by measurements on single filaments. Specifically, pulling on formin increases the association rate constant for processive assembly of profilin–actin by up to threefold (Courtemanche et al., 2013; Jégou et al., 2013). In contrast, pulling on formin has been observed to slow down filament disassembly (Jégou et al., 2013). The data suggest that pulling on either the FH1 or FH2 domains of a formin bound to terminal barbed-end subunits facilitates the structural transition of the formin-barbed end complex towards the 'open state'. Anchored formins have also been shown to exert pico-Newton forces on a depolymerizing filament (Jégou et al., 2013). These findings clearly show that formins are mechanosensitive regulators of barbed-end growth and that they also might apply forces on filaments. Further studies should reveal how these effects vary quantitatively among different formins and how this correlates with their specific biological functions and the structural properties of their FH2 and FH1 domains.

Regulation of barbed-end dynamics by profilin – potential consequences

Profilin binds to the barbed face of actin, which is exposed on monomeric actin (i.e. G-actin) and at the two terminal subunits of the barbed end of the actin filament (F-actin). Profilin has a 100-fold higher affinity for G-actin (K_d=0.1 μM) than for F-actin (K_d≈25 μM), which makes profilin-bound G-actin the main polymerizable actin monomer as it is more abundant in cells than free G-actin (Jégou et al., 2011; Kinosian et al., 2000). Binding of profilin to barbed ends has been shown to enhance the rate of filament disassembly by one order of magnitude *in vitro* (Bubb et al., 2003; Jégou et al., 2011). However, whether this property of profilin is physiologically relevant is questionable, as it is generally thought that all profilin is bound to G-actin in cells (Kaiser et al., 1999; Xue and Robinson, 2013). High concentrations of profilin (10 to 100 μM), obtained either by microinjection or by overexpression, have however been reported to inhibit cell migration and cause disappearance of filaments in lamellipodia (Cao et al., 1992; Joy et al., 2014; Rotty et al., 2015). In the current view, increasing the cellular concentration of profilin should result in filament

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disassembly (to give a concentration of the profilin–actin complex of about 45 µM) and thus should enhance local actin-based motile processes rather than inhibit them. Specifically, formin-bound filaments should grow at 6 µm/s in the presence of 50 µM profilin (assuming a typical on-rate constant of profilin–actin of 50 $\mu M^{-1}\ s^{-1}$) (Jégou et al., 2013). Along this line of reasoning, it is difficult to interpret *in vivo* effects of profilin within models that have been derived from *in vitro* data obtained at a controlled high concentration of free profilin. Clearly, the mechanism by which profilin inhibits motility requires further detailed investigations, following recent *in vitro* approaches (Pernier et al., 2016).

Regulation of nucleation of new barbed ends by WH2 domain proteins

The role of the WH2 domain in the regulation of barbed-end reactivity through capping (Spire) or tracking (Ena or VASP family proteins, VopF) has been mentioned above. The WH2 domain is a versatile actin-binding module and displays multifunctional regulation of actin assembly when expressed in tandem repeats. Spire, Cobl and VopF or VopL nucleate and sever actin filaments, and sequester G-actin *in vitro*. Under physiological conditions, profilin is bound to G-actin and acts as an efficient and high-affinity competitor of WH2-domain-binding to G-actin, making nucleation of filaments from complexes between WH2-repeats and G-actin unlikely. This leaves filament-severing and barbed-end binding as the more physiologically relevant activities of the WH2 domain. Consistent with this idea, VopF localizes at the tips of protrusions induced in VopF-transfected cells, and Spire activates Fmn2 only in its barbed-end-bound form (Montaville et al., 2015; Tam et al., 2007).

The WH2 domain is also present in proteins of the WASP family, which do not directly nucleate filaments, but catalyze filament branching with the Arp2/3 complex (see poster). Filament growth and branching initiate assembly of a polarized branched meshwork that produces force in motile processes, such as cell protrusion, vesicle scission, pathogen propulsion, internalization of endocytic vesicles (see Collins et al., 2011; Mooren et al., 2012; Rotty et al., 2013 for reviews) and, as recently shown, expulsion of exocytic material at the apical plasma membrane (Tran et al., 2015). In the branching reaction, Arp2/3 binds WASP (also known as WAS) at a domain (CA) adjacent to the WH2 domain, with the WH2-CA domain being sufficient for branching. The ability of WH2 to bind actin is required for branching. In the absence of Arp2/3, the WH2 domain of WASP associates with filament barbed ends (Co et al., 2007). At each branching cycle, association of WASP–Arp2/3 to an elongating mother filament leads to incorporation of Arp2/3 into a branch junction from which a daughter filament barbed end elongates. The pushing force produced by filament barbed-end growth against the membrane is thought to be dependent on the duplication of growing filaments at each branching event. We know that only a few filaments are sufficient to propel the baculovirus (Mueller et al., 2014). Hence, understanding the mechanics of force production linked to branching and barbed-end growth at the molecular scale is an important unsolved issue. The dynamics of barbed-end capping in the vicinity of the membrane is regulated by CPI-containing proteins (see above). Thus, the cycle of filament branching, growth and capping appears to be controlled as a whole functional unit.

Live imaging of branched filament assembly *in vitro* using total internal reflection fluorescence (TIRF) microscopy has shown that branching following the association of a WASP–Arp2/3–actin complex with the side of a filament that has been immobilized on a coverslip occurs with a very low frequency (Smith et al., 2013). In comparison, high branching frequencies can be estimated from the rates at which new filaments are generated at the leading edge in live cells. In conclusion, a clear mechanism of filament branching is not yet emerging from currently available *in vitro* and *in vivo* data.

In the cell, filament branching is downregulated by several means. Screens for inhibitory chemical compounds have identified CK-666 and CK-869, which can be used as tools to study branching and Arp2/3 function (Hetrick et al., 2013). In addition, proteins such as Gadkin (also known as AP1AR) or Arpin (Gorelik and Gautreau, 2015; Maritzen et al., 2012) have been found to sequester Arp2/3 into a non-branching complex by mimicking and competing with the C-terminal region (CA) of WASP proteins.

Profilin also inhibits filament branching *in vitro* (Machesky et al., 1999; Rotty et al., 2015; Suarez et al., 2015); this inhibition was measured *in vitro* in conditions where free profilin was present in an excess over profilin–actin, and was proposed to account for the inhibition of cell migration and the disappearance of the lamellipodial filament array at high concentration of profilin (Cao et al., 1992; Joy et al., 2014). However, as discussed above, *in vitro* and *in vivo* situations cannot be easily compared. In the cellular context, an excess of profilin is expected to convert into an excess of profilin–actin. Hence, how profilin inhibits filament branching in lamellipodia remains elusive within the current view of the distribution of free G-actin, free profilin and profilin–actin in live cells. Resolving this issue requires further investigations of the mechanisms that control actin homeostasis, using more integrated, biomimetic, *in vitro* thermodynamic and kinetic assays of actin assembly, following our recent work (Pernier et al., 2016).

Indirect regulation of barbed-end growth by ADF and cofilin family proteins

This poster article focuses on kinetic regulation of actin polymerization by regulatory proteins that directly bind filament barbed ends and control their assembly or disassembly kinetics. However, a number of other actin-binding proteins indirectly participate in the regulation of barbed-end assembly. For instance, proteins belonging to the actin depolymerizing factor (ADF) or cofilin family binds cooperatively to the side of ADP-bound F-actin filaments (Ressad et al., 1998) and destabilize the filament structure (McGough et al., 1997; Ressad et al., 1998). This results in filament severing (Andrianantoandro and Pollard, 2006; Maciver et al., 1991), as well as an enhanced rate of filament disassembly, which is restricted to disassembly from the ADP-bound pointed end in a steady-state solution of F-actin. Increasing the number of filaments by severing in itself cannot affect the stationary pool of monomers in an F-actin solution at steady state, hence does not change the rate at which barbed ends grow. In contrast, an enhanced rate of filament disassembly causes a measured increase in the pool of monomeric actin. The latter effect is responsible for enhanced growth of individual filament barbed ends, which supports motility. Recently, other factors such as Aip1 (also known as DAB2IP), coronin proteins, twinfilin proteins and cyclase-associated proteins (CAP, Srv2 in yeast) have been reported to further enhance filament disassembly in synergy with ADF and cofilin proteins (Gressin et al., 2015; Johnston et al., 2015; Kueh et al., 2008; Mikati et al., 2015; Nadkarni and Brieher, 2014). These effects, however, do not appear to involve barbed-end capping by these proteins, in contrast with what has been previously suggested (Okada et al., 2002).

Conclusions and perspectives

Polarized growth of actin filaments is regulated by barbed-end-binding proteins that either cap or processively track barbed ends, thus either blocking or facilitating the assembly of polarized

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filaments. Regulation of the barbed end is further enforced by a finely tuned control of the concentration of active barbed-end regulators through inhibition (sequestration) or local activation. Many barbed-end regulators either directly compete for binding barbed ends or displace each other from barbed ends through simultaneous transient binding to barbed ends. Actin-binding motifs that are present in WH2 domains, FH2 domains and in various capping proteins mediate this process. Profilin remains intriguing with regard to its role in actin regulation as it binds both to monomeric G-actin and F-actin subunits at barbed ends. Unveiling the interplay of regulatory proteins at barbed ends opens new avenues that will hopefully allow us to quantitatively model actin-based motility.

Competing interests

The authors declare no competing or financial interests.

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Summary: Assembly of actin filaments at barbed ends drives motile processes. Here, we discuss the control of polarized filament assembly by various regulators that interact with and compete for barbed ends.