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Control of Actin Filament Dynamics at Barbed Ends by WH2 Domains: From Capping to Permissive and Processive Assembly

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WH2 domains are multifunctional regulators of actin assembly that can either sequester G-actin or allow polarized barbed end growth. They all bind similarly to a hydrophobic pocket at the barbed face of actin. Depending on their electrostatic environment, WH2 domains can nucleate actin assembly by facilitating the formation of prenuclei dimers along the canonical spontaneous assembly pathway. They also modulate filament barbed end dynamics in a versatile fashion, acting either as barbed end cappers or assisting barbed end growth like profilin or uncapping barbed ends and potentially mediating processive elongation like formins when they are dimerized. Tandem repeats of WH2 domains can sever filaments and either remain bound to created barbed ends like gelsolin, or strip off an ADP-actin subunit from the severed polymer end, depending on their relative affinity for terminal ADP-F-actin or ADP-G-actin. In summary, WH2 domains recapitulate all known elementary regulatory functions so far found in individual actin-binding proteins. By combining different discrete sets of these multifunctional properties, they acquire specific functions in various actin-based processes, and participate in activities as diverse as filament branching, filopodia extension, or actin remodeling in ciliogenesis and asymmetric meiotic division. They also integrate these functions with other actin-binding motifs present either in the same protein or in a complex with another protein, expanding the range of complexity in actin regulation. The details of their molecular mechanisms and the underlying structural basis provide exciting avenues in actin research. © 2013 Wiley Periodicals, Inc.

Key Words: WH2 domain, actin, filament barbed end assembly

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Introduction

Polarized growth of actin filaments at their barbed ends, mediated by regulated treadmilling, is the common feature of actin-based motile processes. Nucleation of actin filaments and subsequent elongation at barbed ends, therefore, have to be exquisitely regulated to adapt the spatial and temporal diversity of kinetics of actin assemblies that occur simultaneously in a living cell [Campellone and Welch, 2010]. Barbed end growth is modulated in two ways, first by proteins that bind monomeric actin in a complex that associates with filament barbed ends with rate parameters that may differ from those of free monomers, second by proteins that associate with filament terminal subunits and modify the structure and reactivity of the barbed ends. These two classes of regulators were initially respectively represented by profilin and capping proteins (CPs), which are widespread and abundant regulators of actin dynamics. Later on, new proteins were discovered that regulate barbed end nucleation, growth, and depolymerization. These include formins and WH2 (Wiskott-Aldrich Syndrome protein (WASP) Homology 2) domains either in single module or in tandem repeats. Intriguingly, these new regulators often combine to various extents some functional properties of profilin and some others of cappers, and further bind actin in a nucleotide-dependent fashion, thus expanding the range of subtleties in the control of actin dynamics. Structural studies of their complexes with monomeric actin indicate that they bind at the barbed face of actin in more or less mutually exclusive fashion, hence may also compete for binding to filament barbed ends. The accumulated biochemical knowledge on the regulation of polarized filament growth opens awaited avenues for further structural studies of the potential multiple conformations of the actin subunits at barbed ends induced by ATP, ADP, and various regulators of barbed end assembly dynamics. Recent advances in cryo-electron microscopy and tomography of F-actin [Oda et al., 2009; Fujii et al., 2010] as well as molecular dynamics simulations [Splettstoesser et al., 2011] should provide important insights in this issue.

Barbed End Assembly Regulation by Profilin, CPs, and Formins

Profilin transiently associates to elongating barbed ends in physiological conditions, CPs are in a more or less slow association–dissociation equilibrium with barbed ends and formins track and assist barbed end growth. These three proteins display overlapping binding sites at the barbed face of actin and functionally compete for barbed ends.

Profilin

Profilin binds MgATP-G-actin with 10^7 M^{-1} affinity [Perelroizen et al., 1994] without preventing its association with filament barbed ends, with almost identical rate parameters, while preventing association to pointed ends [Pollard and Cooper, 1984]. Profilin thus makes filament assembly fully directional and enhances the processivity of treadmilling. The affinity of profilin for ADP-bound F-actin at filament barbed ends is almost two orders of magnitude lower than for ATP-G-actin [0.5 10^5 M^{-1}, Pantaloni and Carlier, 1993]. However, binding of profilin enhances by seven-fold the rate of filament endwise depolymerization [Kinosian et al., 2002; Romero et al., 2007; Jégou et al., 2011], suggesting that profilin destabilizes the bonds between terminal subunits at the barbed ends. This effect is observed for ADP-Pi as well as ADP bound terminal subunits [Jégou et al., 2011; Jégou et al., 2013]. Which of the lateral or longitudinal actin–actin bonds that define the filament helical structure is most affected by profilin binding is not known. The barbed face of actin is exposed on both terminal and penultimate actin subunits at barbed ends. If the actin–actin contacts at the barbed ends are the same as in the core of the filament [Holmes et al., 1990], profilin can bind only the terminal subunit, steric conflict preventing association of a second profilin to the penultimate subunit. Binding of a second profilin molecule has been evoked to account for some biochemical data [Romero et al., 2007]; however, the required structural rearrangements of actin subunits remain an open issue.

Capping Proteins

Capping proteins also bind the barbed face of actin, with an extreme preference for barbed end terminal subunits vs. monomeric actin. Capping totally blocks assembly and disassembly at barbed ends. Practically, all CPs contact actin with the same structural binding element, an amphipathic α-helix whose hydrophobic face recognizes a hydrophobic cleft between subdomains 1 and 3 of actin [Dominguez and Holmes, 2011]. The very high and selective affinity of CPs for the barbed ends (10^9–10^{11} M^{-1}) suggests that, in contrast to profilin, their binding stabilizes actin–actin contacts at the filament end and prevents depolymerization. Gelsolin, the most strongly binding of all CPs known, consists of a repeat of three segments (S1-S2-S3) and (S4-S5-S6), S1 and S4 making similar contacts at the barbed face of actin. Therefore, gelsolin probably binds the two terminal subunits at barbed ends according to a proposed model [Burtnick et al., 1997; Choe et al., 2002] and in agreement with the formation of a tight 1:2 gelsolin$(actin)_2$ complex between gelsolin and G-actin [Bryan and Kurth, 1984; Coué and Korn, 1985]. Capping protein, the homolog of muscle CapZ, is a αβ heterodimer that also interacts with the two terminal subunits at barbed ends [see Cooper and Sept, 2008]. The structure of CP bound to barbed ends derived from cryo-electron microscopy as well as molecular dynamics analysis is the sole structure of a regulated barbed end so far [Narita et al., 2006; Kim et al., 2010]. It reveals that CP too inserts an amphipathic α-helix (the β-tentacle) in the hydrophobic pocket of the terminal subunit, leaving this site unoccupied on the penultimate subunit.

In line with their interaction with the two barbed end subunits, CPs often nucleate pointed end assembly and sometimes sever filaments, remaining bound to the barbed end created by severing. Thus, CP and CapG (a gelsolin-related 41 kDa protein) as well as mammalian twinfilin, which consists of two actin depolymerizing factor (ADF) homology domains, only nucleate filaments [Helfer et al., 2006; Poukkula et al., 2011], whereas gelsolin [Harris and Weeds, 1984], *Dictyostelium* severin [Yamamoto et al., 1982], *Toxoplasma gondii* toxofilin (Carlier, unpublished data), and yeast twinfilin [Moseley et al., 2006] both nucleate and sever filaments. Note that the pointed end nucleating activity of strong cappers, which is observed in vitro in the presence of G-actin at a concentration above the critical concentration for pointed end growth, has no physiological relevance in itself, since in the treadmilling context, filaments grow only at barbed ends and pointed ends only depolymerize. However, the facilitated nucleation by CPs generates a large amount of oligomers that are readily detected in critical concentration plots in the vicinity of the critical concentration [Coué and Korn, 1985]. In the physiological context, this pool of capped oligomers might participate in barbed end nucleation via dissociation of the capper and association with a barbed end nucleator. Physiologically relevant nucleators are expected to generate new filaments that elongate at barbed ends, in a spatially controlled fashion. For nucleators to be effective, capping of barbed ends has to be overcome. Nucleators thus often interact with barbed ends in a way that permits assembly by protecting them from blockage by cappers. This is what formins and WH2 domain proteins do.

Formins

Formins are a family of proteins subdivided in several classes that differ regarding their mode of regulation by small GTPases and are involved in a variety of actin-based processes [Rose et al., 2005; Goode and Eck, 2007; Dominguez, 2009; Schönichen and Geyer, 2010; Maiti et al.,

2012, Breitsprecher and Goode, 2013]. All formins are dimeric polypeptides generally comprising formin homology domains 1 (FH1) and 2 (FH2), which respectively bind profilin and actin. Profilin is generally essential for the in vivo function of formins. The constitutively active FH1-FH2 dimers both nucleate actin filaments to various extents and remain bound to the growing/disassembling barbed ends, hence are considered as processive motors of assembly. Detection of the processive tracking of barbed ends by fluorescence microscopy is possible only for highly processive formins that individually remain bound over several hundred rounds of assembly/disassembly, like yeast Cdc12, Bni1, or mDia1 [Kovar and Pollard, 2004; Romero et al., 2004]. The processivity of other formins, whose FH2 domain binding mode and nucleating efficiency often differ from Bni1 [Thompson et al., 2013] has not been established unambiguously. Formins generally initiate Rho or Cdc42-site-directed barbed end insertional assembly of the helical filament from membrane loci and use their mechanical properties to wind around the filament as it grows [Mizuno et al., 2011]. They affect polymerization and depolymerization in a force-sensitive fashion [Jégou et al., 2013]. The crystal structure of tetra-methylrhodamine-actin in complex with the FH2 domain of Bni1 shows that the FH2 dimer embraces up to three actin subunits arranged in a flat ribbon resembling a pseudo-filament, in which the last two terminal subunits block barbed end growth [Otomo et al., 2005]. Blockage is in part linked to binding of a small alpha-helical secondary structure of the FH2 domain, called the "knob," to the hydrophobic cleft at the barbed face of actin between subdomains 1 and 3, which is also the binding site of CPs. Alternating switches between a blocked and an open state linked to polarized displacement of one FH2 protomer accommodate processive elongation of the FH2-bound barbed end. Profilin plays an important role in the function of formin, since filament assembly is enhanced by FH1-FH2 in the presence of profilin [Kovar and Pollard, 2004; Romero et al., 2004; Shimada et al., 2004]. Conversely, filament disassembly is often blocked by FH2 and allowed by FH1-FH2 in the presence of profilin [Jégou et al., 2013]. Moreover, applying pulling force to either the FH1 or the FH2 domain of barbed-end bound FH1-FH2 of mDia1 identically enhances barbed end growth from profilin–actin, suggesting that a functional complex of profilin–actin with FH1-FH2 is bound to barbed ends. The structure of this complex, which is functionally relevant since profilin is required for formin function, is not known. Since profilin binding to actin is highly dependent on the bound nucleotide, and profilin does not allow filament assembly from ADP-actin, one anticipates that the structural changes in formin-bound barbed end subunits are influenced by ATP/ADP, in a manner that favors processive behavior in relation with ATP hydrolysis.

In summary, profilin/formins and CPs functionally antagonize and compete for binding to barbed ends. Hence, the amount of assembled F-actin at steady state depends on the relative reduced concentrations (free concentration divided by Kd) of these two proteins. These quantitative aspects have to be considered in elaborating reconstituted assays mimicking the cellular contents.

It would be difficult to finely tune actin response to signaling and environmental changes with these sole "open or close" dictators. The WH2 domains solve this problem by their versatility and multifunctional properties. Not surprisingly, they appear to regulate actin dynamics in processes that require a more graded response.

Barbed End Regulation by WASP-Homology 2 (WH2) Domains

WH2 domains are actin-binding protein motifs of about 30 amino acids, which are inserted, either as single modules or as tandem repeats, in proteins that exert various functions in actin-based motile processes [see Dominguez, 2009; Carlier et al., 2011; Kessels et al., 2011]. Although quite a few WH2 domain-containing proteins are known and have been examined experimentally, establishing a common functional role of the WH2 domain(s) remains an elusive issue due to the large variety of their biological activities. Additionally, WH2 domain-containing proteins often interact not only with actin but also with other proteins in large complexes that orchestrate highly integrated processes, in which the WH2-actin interaction is embedded. For instance, WASP family proteins, which are the founding members for the name "WASP-Homology 2" harbor either one or two WH2 domains and also interact with Arp2/3 complex via other regions of the WASP molecule. The 1:1:1 complex of N-WASP, Arp2/3, and actin [Gaucher et al., 2012] associates with an actin filament to initiate branching out of a daughter filament. The growth of branched actin meshworks is responsible for membrane deformations in lamellipodial protrusion, endosomal vesicle fission, or Golgi reorganization. Another example is the Spire protein, which can bind actin via four WH2 domains and by itself caps barbed ends and blocks their growth from profilin–actin [Bosch et al., 2007]. In contrast, when Spire interacts with formin 2 (Fmn2), a non-Diaphanous-related formin, via its N-terminally adjacent KIND domain, massive actin assembly is steered in meiotic asymmetric division [Pfender et al., 2011; Vizcarra et al., 2011; Zeth et al., 2011]. Alternatively, WH2 domains are covalently inserted in proteins that contain other actin-binding domains. Their inclusion generates more complex functions in actin assembly. For instance, leiomodin is a variant of tropomodulin, a protein that associates with tropomyosin to cap pointed ends. Addition of a WH2 domain to the other domains of tropomodulin generates a filament nucleating activity in leiomodin [Chereau et al., 2008]. Similarly, an additional WH2 domain is inserted in formins INF2, FMNL2, and

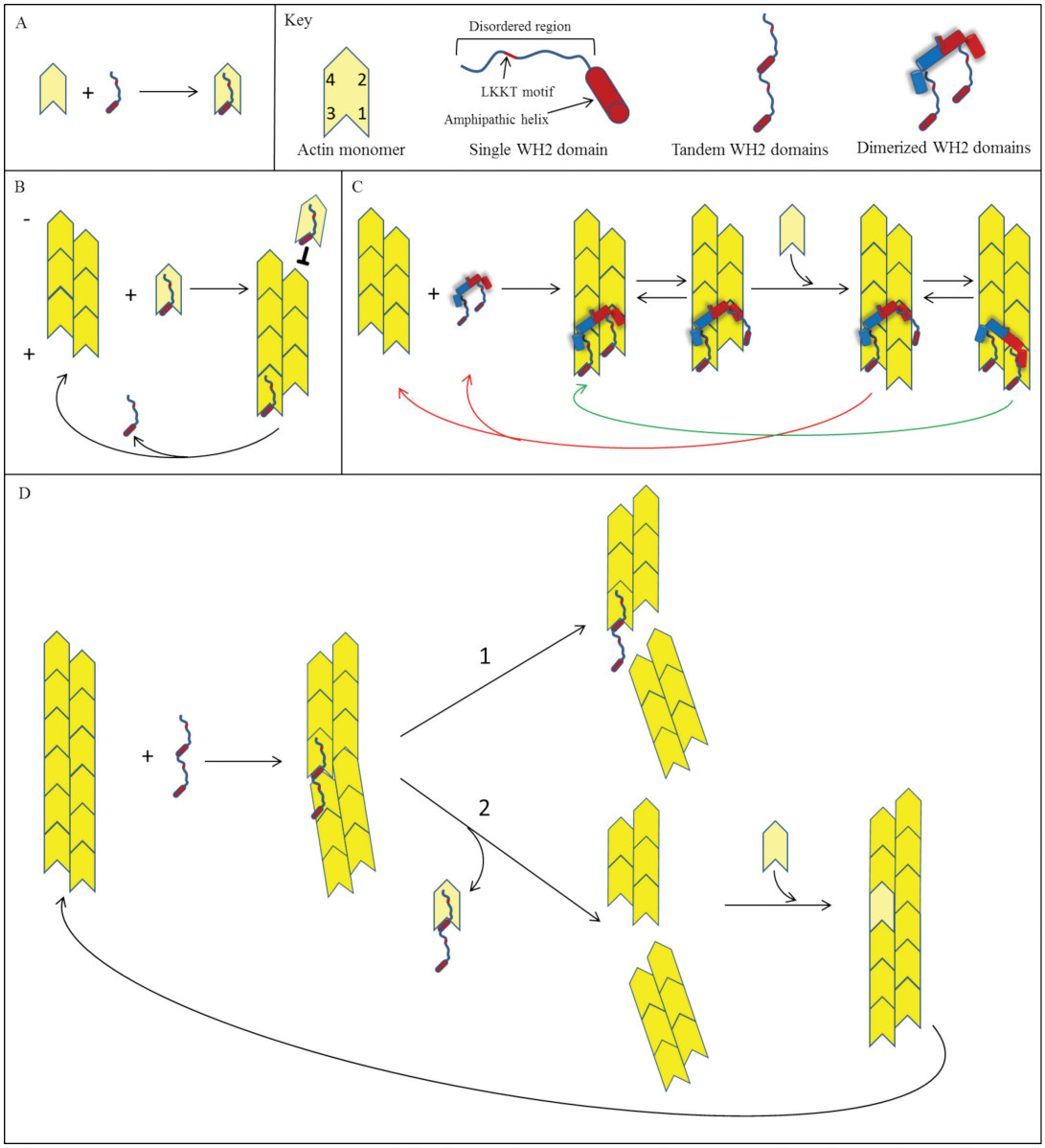

Fig. 1. Multiple regulation of barbed end assembly–disassembly by WH2 domains. **(A)** WH2 domain binding to G-actin. The N-terminal amphipathic α-helix (red cylinder) associates with the hydrophobic pocket at the barbed face of actin between subdomains 1 and 3. The more disordered C-terminal moiety (blue line) binds actin along the side of subdomain 1. The consensus actin-binding sequence LKKT is drawn in red. **(B)** Profilin-like function of WH2: The WH2-ATP-G-actin complex associates to barbed ends exclusively, supporting polarized growth; WH2 enhances the rate of filament barbed end disassembly. **(C)** Barbed end tracking and assisted elongation by dimerized WH2 domains (VASP, VopF/VopL). Green arrow specifies the cycle of processive elongation in which the same molecule of WH2 dimer remains bound to the barbed end through consecutive assembly of actin or profilin–actin. Red arrow specifies the cycle of nonprocessive, assisted elongation in which the WH2 dimer is in rapid association–dissociation with barbed end terminal subunits. **(D)** Low affinity filament side binding of tandem repeat WH2 domains, leading to severing and (i) barbed end capping (route 1): WH2 domain remains bound to the barbed end, blocking growth and depolymerization (case of Spire); (ii) severing and stripping off an ADP-bound actin monomer from the filament (route 2), creating two noncapped filaments that can reanneal (case of COBL). In the latter case, each severing event causes depolymerization and sequestration of one molar equivalent of WH2-ADP-G-actin per cut. Nucleotide exchange on WH2-actin is greatly slowed down. Repeated cycles of severing and reannealing maintain a stationary number of short filaments as long as enough WH2 domains remain free.

FMNL3 [for review on the various formin families, see Breitsprecher and Goode, 2013]. This additional WH2 domain induces a filament severing activity of INF2 [Chhabra and Higgs, 2006] and enhances the processive activity of FMNL3 [Heimsath and Higgs, 2012].

The fact that WH2 domain-containing proteins play a role in important biological processes fosters an increasing number of structural-functional studies of the WH2-actin interaction.

In isolated form, all WH2 domains bind monomeric actin (G-actin) in a 1:1 complex (Fig. 1A). They generally

display a higher affinity for ATP-G-actin than for ADP-G-actin [Paunola et al., 2002]. The values of the equilibrium dissociation constant of WH2-ATP-G-actin complexes lie in the 0.1–1 μM range. WH2 domains all display the same actin-binding mode as the N-terminal and central regions of ß-thymosins [Paunola et al., 2002; Hertzog et al., 2004; Chereau et al., 2005; Dominguez, 2009]. The main binding structural element is, again like for CPs, an amphipathic β-helix that binds the hydrophobic pocket at the barbed face of actin. Like ß-thymosins, WH2 domains compete with profilin for binding to actin. Yet, the WH2-actin 1:1 complexes have a versatile function in actin filament assembly. Either they are nonpolymerizable like β-thymosin-actin complexes, or they participate in polarized barbed end assembly like profilin–actin complex (Fig. 1B). The origin of the switch between these opposite functions resides in subtle differences in electrostatic contacts between discrete residues of WH2 and actin [Hertzog et al., 2004; Didry et al., 2012] (for details see the article by Louis Renault in this issue). Similar differences may be amplified in tandem repeats of WH2 domains, thus contributing in their expanded multifunctional regulation of actin assembly.

WH2 repeat proteins so far include eukaryotic proteins Spire (four WH2), Cordon-Bleu (COBL; three WH2), the WASP family proteins N-WASP and JMY (two WH2) and bacterial effectors of *Vibrio cholerae* and *Vibrio parahaemolyticus*, VopF and VopL, respectively (dimers of three WH2). Spire and COBL regulate actin assembly in developmental processes [Wellington et al., 1999; Carroll et al., 2003; Le Goff et al., 2006; Ravanelli and Klingensmith, 2011] and in neural processes linked to dynamic remodeling, such as those involved in learning and memory [Schumacher et al., 2004; Ahuja et al., 2007; Kessels et al., 2011]. WH2 repeat proteins have generally been found to nucleate spontaneous assembly of pure actin in vitro. It was then proposed they were also nucleators of actin filaments in vivo. Accordingly, overexpression of COBL or Spire induces cellular protrusions or ruffling, which may be a direct or indirect effect of the overexpression of these proteins.

Biochemical and structural studies of the binding of WH2 repeats to actin and of their effect on actin assembly somewhat questioned the view that they merely work as actin nucleators in vivo and suggest they rather act as new regulators of filament barbed end assembly, with specific features that vary from one protein to the other, as follows.

Spire as a Weak Barbed End Capper

Spire does nucleate actin assembly in vitro, but binding of G-actin to each of the four WH2 domains makes a nonpolymerizable Spire(actin)$_4$ (SA4) complex. In this complex, the longitudinal contacts between the four actin molecules that are stabilized by the four adjacent WH2 differ from the actin–actin contacts along the long pitch helix of the filament, and cannot accommodate a helical filament strand configuration due to a too short distance between each WH2 [Renault et al., 2008], therefore SA4 does not adopt the structure of a template filament nucleus [Bosch et al., 2007; Sitar et al., 2011]. Remarkably, Spire also binds barbed ends in a manner that slows down both assembly and disassembly of actin and blocks assembly from profilin–actin, the physiological form of G-actin [Bosch et al., 2007]. Hence, in vivo Spire is unlikely to act as a nucleator, nor as a sequesterer of G-actin, due to efficient competition of profilin. Spire rather may act as a regulator of barbed end dynamics in competition and/or cooperation with formin 2. Spire was also found to sever filaments in a manner reminiscent of gelsolin, that is the severing reaction facilitates its binding to the barbed end created by severing, thus preventing reannealing [Bosch et al., 2007; Chen et al., 2012] (Fig. 1D, route 1). Although these data provide some insight into the function of Spire, we still do not understand how Spire is bound to barbed end terminal subunits. In the standard Holmes model of F-actin [Holmes et al., 1990; Splettstoesser et al., 2011], only the terminal and penultimate actin subunits expose their barbed face to the solvent at the barbed end, enabling them to each associate with a single WH2 domain. For structural reasons, two adjacent WH2 domains cannot interact with two actin subunits belonging to opposite strands of the long pitch helix of the filament, but can partially associate with two adjacent subunits along the same strand, which is the first step in severing (Fig. 1D). If we assume that up to four consecutive actin subunits along each strand of the long pitch helix interact with the four WH2 domains of Spire, up to two Spire molecules might be bound to the barbed end, each of them on one strand. However, in this picture, only the terminal subunits of each strand expose the hydrophobic pocket at their barbed face for WH2 domain binding. Conventional binding of the four WH2 domains of each Spire molecule, therefore, requires disruption of three regular longitudinal actin–actin bonds along each strand of the long pitch helix and should strongly affect the organization and connectivity of the eight terminal subunits. High-resolution electron microscopy and molecular dynamics, together with functional assays using mutated and truncated variants of Spire, and FRET measurements using genetically encoded click chemistry to label Spire at specific positions [Tyagi and Lemke, 2013] may improve our understanding of the structure of Spire-bound barbed ends.

The fact that Spire specifically binds formin 2 [Quinlan et al., 2007], another barbed end binding protein, opens the possibility for either antagonistic or synergistic regulation of polarized actin assembly by these two proteins together. This issue awaits further detailed physical-chemical analysis of the functional interaction between Spire and formin 2.

Cordon-Bleu as an Efficient Dynamizer of Actin Assembly

Although COBL, with three adjacent WH2 domains, displays a structural organization very similar to Spire, and

nucleates spontaneous assembly of pure actin like Spire, its functional profile is strikingly different from Spire's. COBL uses a different set of elementary properties of WH2 domains [Carlier et al., 2011; Husson et al., 2011]. Only the first two of the three WH2 domains binds actin with significant affinity. Two functional adjacent WH2 domains are required but not sufficient for highly efficient severing of filaments. A N-terminally adjacent lysine-rich 16 amino-acid sequence is also required both for nucleation and severing. The WH2 repeats in themselves actually have no nucleation activity, whereas when associated with the lysine-rich region, the first WH2 domain nucleates actin assembly as efficiently as the repeats. In contrast to Spire, COBL does not cap barbed ends. Filament severing is initiated by side binding of COBL to two adjacent F-actin subunits along the filament, followed by disruption of longitudinal contacts and subsequent dissociation of COBL from F-actin as a tight COBL-ADP-actin complex (Fig. 1D, route 2). COBL-severed filaments, in contrast to the Spire-severed filaments, may reanneal (Jiao et al., manuscript in preparation). Additionally, like profilin, COBL binds ATP-G-actin in a complex that feeds barbed end growth. Like profilin again, COBL enhances the rate of barbed end disassembly. Altogether these properties promote a highly dynamic polymerization regime, in which COBL nucleates, supports barbed end growth, multiplies functionally active filament barbed ends by severing, and transiently sequesters a pool of COBL-ADP-actin, that waits for COBL dissociation and ATP exchange for bound ADP to regenerate polymerizable actin. These ingredients are sufficient to establish an oscillatory polymerization regime reminiscent of the dynamic instability-driven oscillatory polymerization of microtubules [Carlier et al., 1987]. Whether and how these properties of COBL are used in vivo, in regulating actin assembly in ciliogenesis, is a burning issue [Ravanelli and Klingensmith, 2011]. In a physiologically relevant context where profilin–actin is the main polymerizing G-actin species, COBL, like Spire, is less likely to nucleate actin assembly. Its main properties are restricted to filament severing without capping barbed ends, which facilitates its potential role in rapid remodeling of the actin cytoskeleton.

An important consequence of the biochemical analysis of COBL is that a single WH2 domain, in the appropriate electrostatic environment (here the adjacent lysine-rich region) is sufficient to nucleate actin assembly. This result, in addition to the facts that neither the two WH2 domains of N-WASP [Gaucher et al., 2012] nor the three WH2 domains of COBL nucleate actin assembly by themselves, rules out the template nucleation model that was proposed for WH2 repeat proteins [Quinlan et al., 2005; Ahuja et al., 2007]. It further explains why additional single WH2 modules in proteins like *Chlamydia* translocated actin recruiting protein (TARP) [Jewett et al., 2006] or formin FMNL3 [Heimsath and Higgs, 2012] confer or enhance the nucleating activity of the proteins.

Vasodilator-Stimulated Phosphoprotein (VASP) and VopF/VopL as Oligomerized WH2: Potential New Barbed End Regulation as Barbed End Uncappers and Processive WH2 Machines

Vasodilator-stimulated phosphoprotein is the first discovered member of Ena/VASP family proteins which play a positive role in actin-based motility processes, including lamellipodium protrusion [Rottner et al., 1999], filopodia extension [Lebrand et al., 2004], *Listeria* propulsion [Chakraborty et al., 1995; Laurent et al., 1999], cell focal adhesions [Haffner et al., 1995]. The C-terminal region of VASP (EVH2) comprises a WH2-like domain followed by an F-actin-binding region and a coiled-coil region that mediates tetramerization [Ferron et al., 2007; Bear and Gertler, 2009]. Thus, in VASP, the individual WH2-like domains are multimerized on parallel peptides rather than organized in tandem repeats like in Spire or COBL. VASP nucleates actin filaments in vitro [Laurent et al., 1999]. VASP also possesses a barbed end binding activity that protects barbed ends from cappers [DiNubile et al., 1995; Bear et al., 2002; Barzik et al., 2005; Pasic et al., 2008] and confers the protein a barbed end tracking and processive activity in stimulation of filament elongation [Breitsprecher et al., 2008, 2011; Hansen and Mullins, 2010]. The actin-binding mode of VASP in the regulation of barbed end elongation is not understood in detail. Its association with IRSp53 may promote processive elongation of filaments supporting filopodia extension [Krugmann et al., 2001] (Disanza et al., in revision).

Interestingly, a similar organization of WH2 domains in a dimeric structure is found in two bacterial proteins, VopF and VopL (*Vibrio* outer proteins F and L, respectively). VopF and its ortholog VopL are pathogen effectors that are injected in host eukaryotic cells by the T3SS secretion system of *Vibrio cholerae* and *Vibrio parahaemolyticus*, respectively. Once internalized, VopF and VopL harness the actin cytoskeleton of the host and induce filopodia and stress fibers, respectively [Liverman et al., 2007; Tam et al., 2007]. These proteins are made of three adjacent WH2 domains assembled in a homodimer by a C-terminal dimerization domain, whose crystal structure has been solved in VopL [Namgoong et al., 2011; Yu et al., 2011]. Like other WH2 repeats, they nucleate actin assembly in vitro. Biochemical analysis reveals their multifunctionality. Like COBL and Spire, VopF and VopL sever filaments and bind barbed ends, however, in contrast with Spire, their association with barbed ends does not block filament elongation from profilin–actin [Pernier et al., 2013]. The complex of VopF with G-actin fails to adopt the helical hexameric structure that was proposed as the basis for a filament template nuclation model [Namgoong et al., 2011; Yu et al., 2011]. As already observed on COBL, only two WH2 domains bind actin with significant affinity, the central WH2 domain merely acting as a linker between the first

and the second one. The resulting complex of dimeric VopF with four actins (two per protomer) adopts an elongated shape in which the four actins are not connected by lateral bonds. Thus, as observed with Spire and COBL, the major complexes of actin and VopF are sequestered forms of actin and cannot act as nucleus of a filament. Remarkably, while the monomeric forms of VopF/VopL containing two or three WH2 are able to nucleate actin assembly, the dimeric forms containing either one, two or three WH2 nucleate in a much more efficient manner [Namgoong et al., 2011; Yu et al., 2011; Pernier et al., 2013]. Only the dimeric forms of VopF containing a minimum of one WH2 domain per protomer bind barbed ends without inhibiting growth from actin nor profilin–actin [Pernier et al., 2013]. Remarkably, dimeric VopF constructs not only protect the barbed ends from capping by CP, but cause uncapping. That is, addition of dimeric VopF to a solution of filaments whose barbed end growth has been arrested by CP promotes immediate regrowth. This result indicates that CP, which by itself dissociates from barbed ends with a half time of 20 min, is kicked off the barbed ends very rapidly when VopF binds to CP-bound barbed ends in a transient unstable complex. Formation of the ternary complex of terminal subunits with VopF and CP precedes dissociation of CP and concomitant stabilization of VopF with growth-competent barbed ends. This process implies that a WH2 domain from one VopF protomer can bind to its available site at the barbed face of the penultimate subunit of the barbed end (while the ultimate subunit is bound to the β-tentacle of CP), thus weakening the association of CP to the filament end and promoting uncapping of CP. Subsequent strengthening of the binding of VopF to barbed ends restores barbed end growth. In this process, the two WH2 domains from the two protomers bind the barbed face of the terminal and penultimate subunits and act like a dimeric FH2 domain of formins and track growing barbed ends. Only one WH2 domain per protomer is sufficient for barbed end uncapping and tracking. This novel regulation of barbed end dynamics emerges from the dimerization of WH2 domains and extends their multifunctionality [Pernier et al., 2013]. The pronounced properties of nucleation, barbed end binding and supported barbed end growth of dimeric fragments of VopF are reminiscent of the activities of VASP and suggests that two WH2 domains, organized in a dimeric parallel structure via a C-terminally positioned coiled-coil region, rather than in adjacent repeats on the same polypeptide, could adopt a conformation allowing them to associate each with the barbed face of the terminal and penultimate subunits of a filament barbed end. In this configuration, each WH2 domain would make contacts with the hydrophobic pocket of each of the two terminal subunits at barbed ends (Fig. 1C). Barbed end tracking and processive elongation might as well be a consequence of this configuration, in which cycles of dissociation of only one WH2 domain from the barbed end penultimate subunit, followed by association of an actin subunit would allow persistent binding of the protein to the barbed end during filament growth, in a manner similar to formins. More detailed structural studies are required to analyze these putative configurations of WH2 domains at barbed ends.

Perspectives

Role of WH2 Domains in Actin Filament Nucleation

Although the main complexes formed between WH2 repeat proteins and actin cannot be considered as nuclei of the actin filaments, two facts are undisputable: (1) the individual WH2 domain has the ability to interact with the filament barbed end in a variety of functional ways; (2) WH2 as a single module or in repeats, in an appropriate electrostatic environment and in the presence of an excess G-actin, can nucleate actin assembly. These facts can be accommodated by a general model according to which WH2 domains may interact with and stabilize one of the transient unstable prenuclei of the filament along the regular spontaneous nucleation pathway [Sept and McCammon, 2001] by making electrostatic actin–actin interactions more favorable [Crevenna et al., 2013]. In support to this proposal, the efficiency of nucleation of WH2 repeats is highly pH and ionic strength dependent (Carlier, unpublished). This model produces simulated spontaneous assembly curves that superimpose the experimentally recorded curves in a large range of COBL concentrations, while incorporating the rate and equilibrium parameters measured for COBL interaction with G- and F-actin in ATP/ADP bound forms [Husson et al., 2011].

Functional Variability of WH2 Domain Association with Filament Barbed Ends

All WH2 domains interact with filament barbed ends, more or less strongly and maybe with an affinity that depends on the nucleotide bound to terminal F-actin subunits. The main binding site of WH2 remains the hydrophobic cleft at the barbed face of actin, which is exposed on the terminal and penultimate barbed end subunits (Fig. 1). What sequence elements of WH2 domains determine their strength of capping and potentially their ability to track barbed ends in a more or less processive manner has to be addressed using mutagenetic and domain swapping strategies. The functional information derived from the analysis of a few members of the WH2 repeat protein family is already sufficient to design chimeric or mutated proteins and challenge their function in actin assembly. Of particular interest is the possibility to create processive machines that use dimerized WH2 domains to track barbed ends and assist filament growth with defined kinetic parameters. Engineering modified WASP family proteins that use

foreign WH2 domains of defined biochemical function will probe the role of WH2-actin interaction in the filament branching reaction with Arp2/3 complex. These synthetic proteins will be useful tools to probe or interfere with the function of native WH2 domain proteins in vivo. Finally, since functional variability must be associated with structural variability, the WH2 domains open new questions regarding the modulation of the structural organization of actin subunits at barbed ends.

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