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Minireview

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Regulation of Actin Cytoskeleton Dynamics in Cells

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The dynamic remolding of the actin cytoskeleton is a critical part of most cellular activities, and malfunction of cytoskeletal proteins results in various human diseases. The transition between two forms of actin, monomeric or G-actin and filamentous or F-actin, is tightly regulated in time and space by a large number of signaling, scaffolding and actin-binding proteins (ABPs). New ABPs are constantly being discovered in the post-genomic era. Most of these proteins are modular, integrating actin binding, protein-protein interaction, membrane-binding, and signaling domains. In response to extracellular signals, often mediated by Rho family GTPases, ABPs control different steps of actin cytoskeleton assembly, including filament nucleation, elongation, severing, capping, and depolymerization. This review summarizes structure-function relationships among ABPs in the regulation of actin cytoskeleton assembly.

INTRODUCTION

Cell movement is a vital phenomenon in most biological processes, including embryonic morphogenesis, immune surveillance, angiogenesis and tissue repair and regeneration (Hussey et al., 2006; Itoh and Yumura, 2007; McMahon and Gallop, 2005; Puppo et al., 2008; Yamaguchi and Condeelis, 2007). Actin cytoskeleton dynamics plays a crucial part in most of these processes, mediating the formation of cellular structures such as lamellipodia, filopodia, stress fibers and focal adhesions (Bailly and Condeelis, 2002). A characteristic feature of all these processes is the dynamic transition of the cellular actin between its monomeric (G-actin) and filamentous (F-actin) forms (Fig. 1C). Actin is an ATPase and ATP hydrolysis by actin plays an essential role in regulating this transition (Fig. 1A). Thus, the actin filament is asymmetric; actin monomers join the barbed (or +) fast growing end of the filament in the ATP-bound state and depart the filament preferentially from the pointed (or -) end primarily in the ADP state, giving rise to a process known as actin filament threadmilling (Figs. 1B and 1C). In addition, the transition between G- and F-actin is tightly regulated in cells by a large number of G- and F-Actin-Binding Proteins (ABPs) (Fig. 1C).

ABPs carry out a wide range of functions, including actin filament nucleation, elongation, severing, capping, and crosslinking and actin monomer sequestration (Fig. 2) (Pollard and Borisy, 2003). The reorganization of the actin cytoskeleton is regulated in time and space by multiple factor, most notably Rho family GTPases that act as GTP-dependent molecular switches (Raftopoulou and Hall, 2004). Among the small GTPases of the Rho family, Cdc42, Rac, and Rho are recognized as the most important regulators of actin assembly, controlling respectively the formation of filopodia, lamellipodia, and stress fibers (Etienne-Manneville and Hall, 2002). Signals transmitted through these GTPases lead to localized actin cytoskeleton assembly/disassembly at the plasma membrane, with the actin filaments acting to push the cellular membrane (Fig. 2) (Hall, 1994). Generally, ABPs are modular polypeptides, which in response to signaling cues undergo conformational changes, and further transmit these signals to downstream cytoskeletal partners and membranes. This is the case for example with the well-studied open/closed transition of the Arp2/3 complex Nucleation Promoting Factor (NPF) protein WASP (Figs. 3 and 5) (Takenawa and Suetsugu, 2007). The general outcome from these mechanisms is rapid changes in actin polymerization/depolymerization near the plasma membrane. Therefore, proteins such as the Bin/Amphiphysin/Rvs (BAR) family that link the cytoskeleton to signaling and membranes have lately attracted significant attention (Fig. 7) (Dawson et al., 2006; Frost et al., 2007; Takenawa and Suetsugu, 2007).

With so many cellular functions depending on the actin cytoskeleton, it is not surprising that abnormal regulation or functioning of cytoskeletal components is often a cause (or a cofactor) of numerous diseases, including cancer, neurological disorders, cardiomyopathies, cholangiocyte, glomerulosclerosis, Wiskott-Aldrich syndrome (Condeelis et al., 2005; Doctor and Fouassier, 2002; Huang et al., 2008; Machesky and Insall, 1998; Myers et al., 2006; Yamaguchi and Condeelis, 2007). While significant knowledge of the mechanisms controlling actin cytoskeleton dynamics has accumulated during the past decade from cellular and biochemical studies, detailed structural information is often lacking. Here, we sum-

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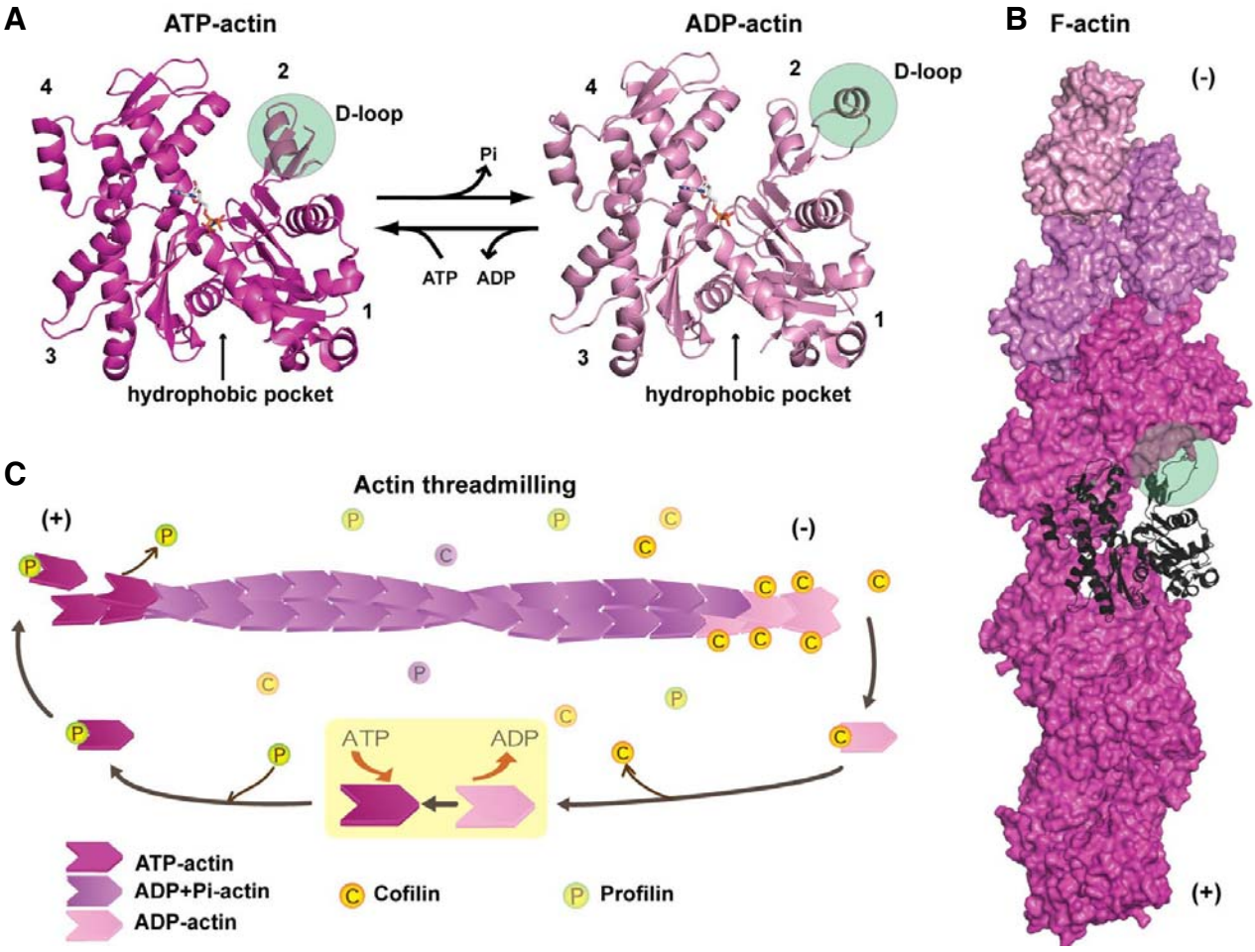

Fig. 1. Monomeric and filamentous actin. (A) The actin monomer consists of two major and structurally related domains, which because of their relative positions within the actin filament are known as the outer and inner domains. The two major domains are in turn subdivided into two subdomains each: subdomains 1 and 2 (outer domain) and subdomains 3 and 4 (inner domain). The combination of these four subdomains gives rise to two prominent clefts. The largest cleft, located between subdomains 2 and 4, houses the nucleotide-binding site. Diametrically opposed to the nucleotide-binding cleft is the so-called hydrophobic or target-binding cleft, which mediates the interaction of actin with most ABPs (Dominguez, 2004). Conformational changes occurring within the actin monomer resulting from the hydrolysis of ATP, translate into changes in the target-binding cleft, which in turn modulates the binding affinities of ABPs and actin itself, leading to changes in the stability of the actin filament. (B) Model of the actin filament (F-actin), resulting from fiber diffraction of oriented actin filaments and fitting of the actin monomer structure (Oda et al., 2009). The D-loop (shaded area in part A), which is typically disordered in most structures of actin, is thought to be one of the major determinants of actin-actin interactions in the actin filament (see monomer represented as a black ribbon), by binding in the target-binding cleft of the actin subunit positioned immediately above it in the filament (Oda et al., 2009). (C) The double-helical actin filament is structurally in kinetically asymmetric, leading to what is known as actin filament threadmilling. Thus, the barbed (or +) end exposes subdomains 2 and 3 of the actin subunits and is characterized by net incorporation of actin monomers in the ATP state. The pointed (or -) end exposes subdomains 2 and 4 of the outermost actin subunits and is characterized by net dissociation of actin monomers in the ADP state. In cells, however, actin filament threadmilling is tightly regulated by ABPs. Thus, ADF/cofilin accelerates the dissociation of actin monomers form the pointed end, whereas profilin accelerates nucletide exchange to promote the incorporation of ATP-bound monomers to the barbed end.

marize recent progress in our understanding of the structure-function of ABPs.

Monomeric and filamentous actin

In cells, actin exists in two states, the monomeric and filamentous states. Actin cytoskeleton dynamics is regulated by controlling the homeostatic balance between these two forms of actins, in response to extracellular stimuli (Fig. 1) (Ridley and Hall, 1992; Ridley et al., 1992). Eukaryotic actin is highly conserved in evolution from yeast to humans. Its atomic structure consists of two major domains, each consisting of two smaller subdomains (Graceffa and Dominguez, 2003; Holmes et al., 1990; Otterbein et al., 2001). According to their arrangement within the actin filament, the two major domains are known as the outer (comprising subdomains 1 and 2) and inner (comprising subdomains 3 and 4) domains (Fig. 1A). The two major domains are structurally related and might have emerged from a gene duplication event early in evolution.

The actin filament is asymmetric, and can be described as either a single left-handed short-pitch helix, with consecutive lateral subunits staggered with respect to one another by half a

monomer length, or two right-handed long-pitch helices of head-to-tail bound actin subunits. Actin is an ATPase and nucleotide hydrolysis by actin helps regulate the transition between its G- and F-actin states in a process known as actin filament threadmilling (Fig. 1C). At steady state and a given G-actin concentration, F-actin grows at the barbed (+) end by spontaneous addition of ATP-bound G-actin and shortens (or depolymerizes) at the pointed (-) end by dissociation of (primarily) ADP-bound G-actin (Pollard et al., 2000).

The asymmetry of the actin filament (or directionality of growth) depends on the polarity of the actin monomers controlled by nucleotide-dependent conformational changes, which induce moderate but accumulatively important changes in the structure and stability of the filament (Belmont et al., 1999; Egelman, 1994; Scoville et al., 2006). Thus, for instance, the DNase I-binding loop (or D-loop) within subdomain 2 plays a critical role in monomer-monomer contacts within the filament. Changes in subdomain 2 and the D-loop occurring during ATP hydrolysis are thought to change the stability of actin monomers in the filament, thus promoting their dissociation (Figs. 1A and 1B) (Egelman, 1994; Holmes et al., 1990; Oda et al., 2009; Otterbein et al., 2001; Schutt et al., 1993; Scoville et al., 2006). However, a high-resolution structure of the actin filament is not yet available, and thus the exact nature of the transition is not fully understood. Since actin has a natural tendency to self-associate under physiological salt conditions, obtaining a homogeneous distribution of actin filaments for crystallization has proven extremely difficult. A potential solution to this problem is being pursued by various laboratories, by using ABPs to build 'rulers' to control the length distribution of actin filaments, and thereby produce monodisperse species for structural analysis.

Rho-family GTPase and the regulation of actin-cytoskeleton dynamics

During the past decade, a key question in the cytoskeleton field has been the identification of specific factors triggering cytoskeleton rearrangement in time and space. Although the molecular mechanisms remain to be fully elucidated, it is now generally accepted that signals received by cell surface receptors *via* chemical messengers such as cytokines, growth factors, and hormones, are transmitted to Rho-family GTPases, in particular Rho, Rac, and Cdc42 (Fig. 2) (Foster et al., 1996; Hall, 1994). The information from specifically integrated signaling pathways is then dispatched to cytoskeleton effector proteins, resulting in a myriad of processes such as acto-myosin movement (Ridley and Hall, 1992), lamellipodia (Ridley et al., 1992) and filopodia formation (Kozma et al., 1995). Consistent with this model, the ectopic expression of dominant negative mutants of Rho-family GTPases in mammalian cells severely impairs actin cytoskeleton dependent processes, including cell migration (Nobes and Hall, 1999), cytokinesis (Mabuchi et al., 1993; Prokopenko et al., 2000), endo/exocytosis (Caron and Hall, 1998), axon guidance (Luo et al., 1997), and morphogenesis during development (Lu and Settleman, 1999; Settleman, 1999). The presence of Rho-family GTPases and their effector proteins at specific loci on the membrane dictates the directionality of cell migration, with the barbed ends of the actin filaments oriented toward the membrane.

Significant effort has been devoted to identifying the specific cytoskeleton effectors of Rho family GTPases. It is well known that a large number of proteins interacting with Cdc42 and Rac contain a short stretch of ~18 amino acids referred to as the Cdc42/Rac Interactive Binding (CRIB) motif (Burbelo et al., 1995). Database searches for CRIB motif-containing proteins have identified a number of potential Rho-family effector proteins, including WASP, formins, and IRSp53 (Burbelo et al., 1995; Yamagishi et al., 2004; Zigmond, 2004b). These proteins are typically modular, i.e. they contain multiple domains, including actin-binding domains such as the WH2 and FH2 domains, and protein-protein interaction or scaffolding modules (Fig. 3). Another common trait is that these proteins are typically self-inhibited by internal interactions, which are commonly released by the binding of the GTPases. Thus, the binding of Rho GTPases accomplishes two major functions, activation and recruitment to specific loci at the membrane.

A well-studied example is the activation of the Apr2/3 complex NPF protein WASP by the Rho GTPase Cdc42 (Takenawa and Suetsugu, 2007) (Fig. 3A). In the resting state, the C-terminal WCA domains of WASP is masked by intramolecular interaction with the CRIB motif located toward the N-terminus of WASP. This conformation is referred to as the closed or auto-inhibited conformation. Upon stimulation, WASP is recruited to the plasma membrane by binding simultaneously to GTP-Cdc42, through its CRIB domain, and to the plasma membrane, through its Basic (positively charged) domain located near the CRIB domain (Prehoda et al., 2000). The activated conformation of WASP, known as the open conformation, releases the WCA inhibition, allowing for the recruitment and activation of polymerization through the Arp2/3 complex.

Formins are thought to be activated in a similar manner (Fig. 3B). Thus, in formins of the mammalian diaphanous (mDia) family, the autoinhibited conformation results from internal interaction between the dimerization domain (DID), located near the N-terminus, and the diaphanous autoregulatory domain (DAD), located near the C-terminus. The binding of GTP-Rho to the G-protein-Binding Domain (GBD), located immediately N-terminal to the DID, releases this inhibitory interaction, freeing the polymerization activity of the formin (Eisenmann et al., 2007; Goode and Eck, 2007; Peng et al., 2003; Watanabe et al., 1997).

Other than Rho GTPases, phosphorylation/dephosphorylation frequently regulates actin cytoskeleton assembly. A well-studied example is cofilin phosphorylation, which abolishes its actin-binding activity, thereby reducing filament breakdown. Ultimately, however, cofilin is also regulated by Rho GTPases, albeit in a less direct way. Thus, Rho regulates cofilin activity *via* Rho kinase (ROCK) that phosphorylates LIM kinase (LIMK), which in turn phosphorylates cofilin (Fig. 2) (Arber et al., 1998; Bamburg, 1999; Edwards et al., 1999; Maekawa et al., 1999). This signal transduction pathway modulates actin assembly in many cell types in response to various extracellular stimuli (Yang et al., 1998; Scott, 2007 #214).

Actin-binding proteins (ABPs)

Dynamic actin threadmilling near the cell membrane is an essential part of cell motility. This process can be subdivided into separate events, including filament nucleation, elongation, depolymerization, capping, severing, crosslinking, and actin monomer sequestration (Fig. 2) (Pollard and Borisy, 2003; Rafelski and Theriot, 2004; Zigmond, 2004a). Each of these events is controlled by specific subsets of ABPs. Structural analysis suggests that ABPs belong to a limited number of folds, including the actin-depolymerizing-factor/cofilin (ADF/cofilin) (Lappalainen et al., 1998), Wiskott-Aldrich syndrome protein (WASP)-homology domain 2 (WH2) (Paunola et al., 2002), gelsolin-homology domain (McGough et al., 2003), calponin-homology (CH) domain (Gimona et al., 2002), formin homology 2 domain (FH) (Goode and Eck, 2007). These proteins can bind G-, F-actin, or both. A special group of ABPs are the members of the myosin superfamily (Sellers, 2000), which are molecular motors that

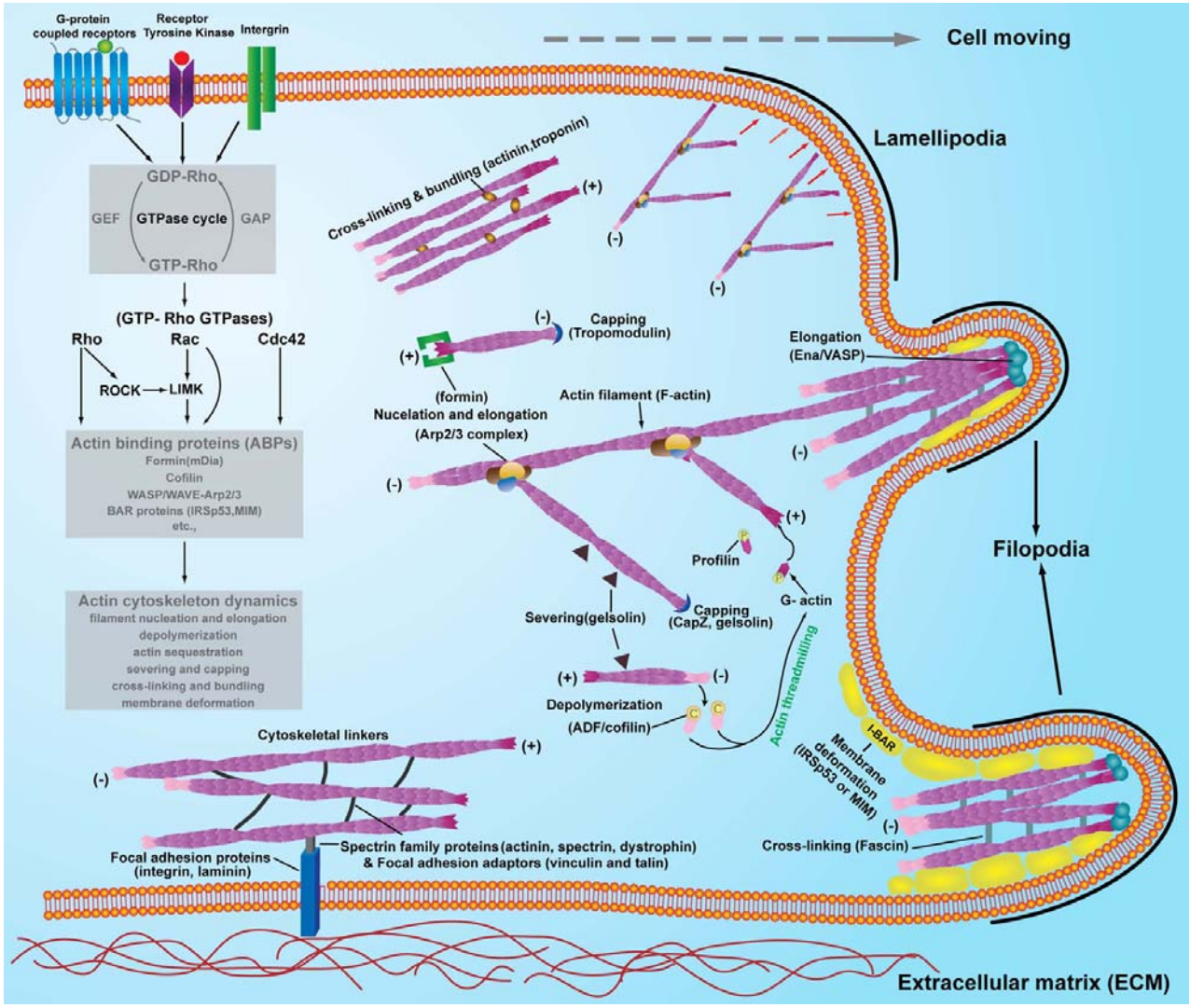

Fig. 2. Different actin filament networks in cells. Rho-family GTPases, including Rho, Rac, and Cdc42, are master regulators of the actin cytoskeleton. Ligand-stimulated transmembrane receptors activate Rho-family GTPases, whose hydrolysis cycle is modulated by guanine nucleotide exchange factors (GEFs) and GTPase activating proteins (GAPs). Activated GTPases (GTP bound form) interact with downstream ABPs effectors that in turn regulate actin filament rearrangements. ABPs can be categorized into families that interact with either monomeric actin (sequestering and depolymerizing proteins) or the actin filament (capping, severing, crosslinking proteins). The combined actions of signaling and ABPs leads to the formation of different cytoskeletal networks, such as focal adhesions, lamellipodia and filopodia. A group of proteins, known as BAR-domain proteins, are emerging as a critical linkage between signaling, the cytoskeleton and cellular membranes.

use F-actin as a track for motility. Many other proteins bind F-actin, and appear to use the actin filaments as a scaffold for their activities.

G-actin-binding proteins

Proteins that bind the actin monomer play a critical role in controlling the pool of unpolymerized actin in cells, by sequestering actin monomers and thereby modulating the pointed and barbed end addition/dissociation of actin monomers. Representative members of this group include the WH2-related protein Thymosin-beta-4 (Tβ4), profilin and ADF/cofilin (Fig. 4). The structures of various complexes of actin with ABPs have been determined (Chereau et al., 2005; Domanski et al., 2004; Hertzog et al., 2004; McLaughlin et al., 1993; Paavilainen et al., 2008; Schutt et al., 1993). A common feature among most of these proteins is the presence of a structurally conserved amphipathic α-helix that binds in the hydrophobic, or target-binding, cleft located between subdomains 1 and 3 at the barbed end of the actin monomer (Fig. 4) (Dominguez, 2004; 2007).

The WH2 domain from diverse proteins, including ciboulot, missing-in-metastasis (MIM), and WASP-family proteins, display a similar architecture consisting of an N-terminal α-helix followed by a extended region featuring the "LKKT(V)" signature sequence motif (Fig. 4A) (Chereau et al., 2005; Dominguez, 2007; Hertzog et al., 2004; Lee et al., 2007). The hydrophobic side of the N-terminal α-helix faces the hydrophobic cleft in the actin monomer. Despite significant length and sequence variability, the C-terminal portions of the WH2 domains of different proteins follow a similar path on the actin monomer, streaming toward

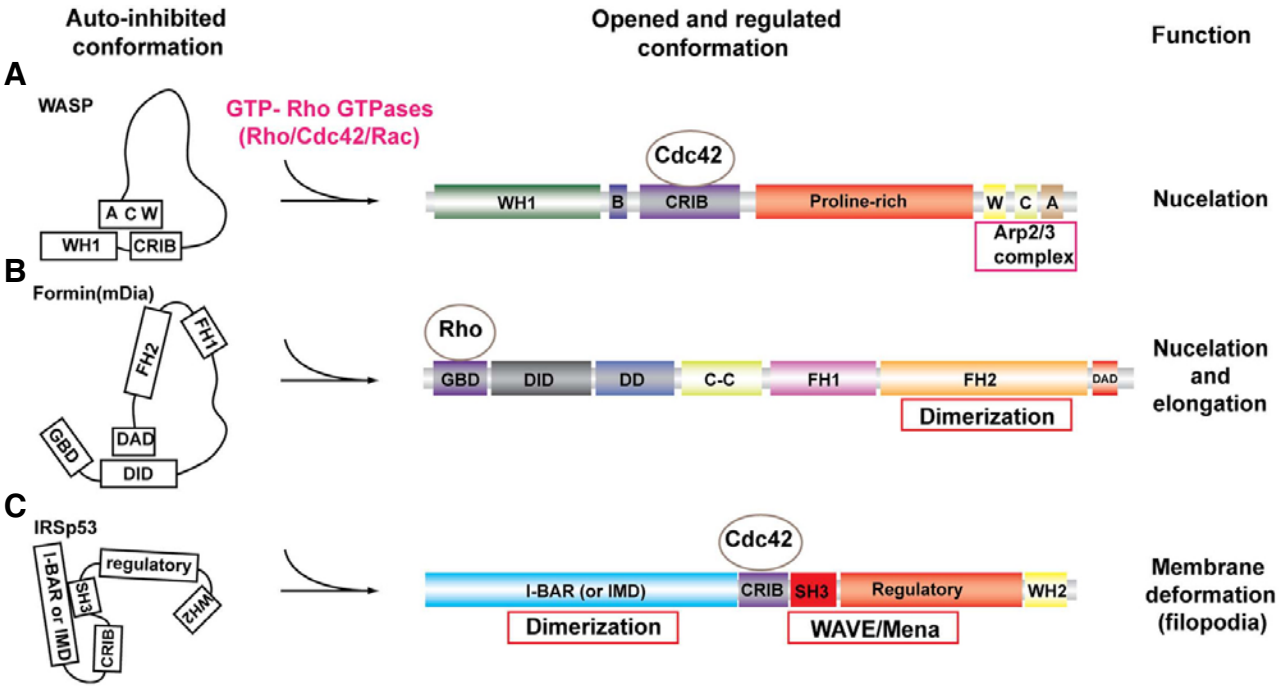

Fig. 3. Internal auto-inhibition and activation by Rho-family GTPases are common themes among cytoskeletal proteins. Internal auto-inhibitory interactions in WASP (A), formin (B), and IRSp53 (C) are counteracted by Rho-family GTPases. In the case of WASP, Cdc42-mediated activation frees the C-terminal CA region to bind and recruit Arp2/3 complex for nucleation (Takenawa and Suetsugu, 2007). Similarly, the Rho-mediated reversal of internal DID-DAD interactions in formin allows the FH2 domain to recruit actin monomers for nucleation and elongation (Eisenmann et al., 2007; Peng et al., 2003; Watanabe et al., 1997). In the case of IRSp53, it is thought that the binding of Cdc42 to the CRIB domain frees the BAR domain to bind membranes while also enabling the recruitment of cytoskeletal effectors via the SH3 domains (Govind et al., 2001; Krugmann et al., 2001).

the pointed end of the actin monomer, i.e. towards subdomains 2 and 4. The interactions of the LKKT(V) motif and the C-terminal extended region of the WH2 domain are for the most part electrostatic in character, with positively charged amino acids of the WH2 domain facing negatively charged amino acids on the actin surface (Fig. 4B).

The structures of actin complexes with the filament capping and severing protein gelsolin (McLaughlin et al., 1993) and the monomer sequestering protein vitamin D-binding protein (DBP) (Otterbein et al., 2002) also show a similar interaction, i.e. both proteins present helices that bind in the hydrophobic cleft of the actin monomer (Dominguez, 2004). Gelsolin helix S70 to N89 and DBP helix S194 to D204 contain hydrophobic residues that bind in the hydrophobic cleft between actin subdomains 1 and 3 (Otterbein et al., 2002). Although the contact interface with actin is much larger for DBP that gelsolin, this helix has been proposed to be the most critical element of their respective interactions with actin (Figs. 4A and 4C). Furthermore, it has been suggested that the existence of this overlapping interaction in two structurally and functionally unrelated proteins allows DBP to sequester actin by forcing gelsolin out of its complex with actin, thereby allowing DBP to proceed with its actin "scavenger" function in the bloodstream (Otterbein et al., 2002). Indeed, owing to its natural tendency to polymerize, the increased concentration of actin monomers in the bloodstream during tissue injury can have devastating effects, and DBP is thought to help remove excess actin from the bloodstream by accelerating its degradation (Janmey et al., 1986).

ADF/cofilin is structurally related to gelsolin (Fedorov et al., 1997; McLaughlin et al., 1993). Early structural and biochemical studies had indicated that members of the ADF/cofilin family, including ADF1 (Bowman et al., 2000), cofilin (Fedorov et al., 1997), and twinfilin (Paavilainen et al., 2002) (Fig. 3A), also contained a helix that bound in the hydrophobic cleft of actin (Dominguez, 2004). This prediction was recently confirmed by the determination of the crystal structure of a complex of actin with twinfilin's C-terminal ADF domain (Figs. 4A and 4C) (Paavilainen et al., 2008), as protein consiting of two ADF/cofilin domains, which binds ADP-actin monomers and filament barbed-end (Helfer et al., 2006; Ojala et al., 2002). In the structure, the helix comprising twinfilin residues I266 to S274 binds in the hydrophobic cleft of actin. Twinfilin residues Q176, at the beginning of the ADF domain, and S274 are highly conserved in the ADF/cofilin family and play critical roles in the interaction with filamentous actin (Grintsevich et al., 2008; Guan et al., 2002; Lappalainen et al., 1997), suggesting that the structure also provides a reasonable model for the interaction of ADF/cofilin with the actin filament (Figs. 3A and 4C).

As illustrated by the examples above, despite the lack of overall sequence similarity among ABPs, the hydrophobic cleft in actin is emerging as the most important determinant of their interactions with actin, which is probably due to the following reasons. First, the hydrophobic cleft on actin displays remarkable plasticity; regardless of the specific amino acid sequence and directionality of binding (inwards or outwards), the helices of the various ABPs discussed above superimpose well in the hydrophobic cleft of actin (Fig. 4B). Thus, for instance, the helices of WH2 domains and ciboulot (Chereau et al., 2005; Dominguez, 2004; Hertzog et al., 2004) run in opposite direction (back to front according to the classical view) to those of gelsolin

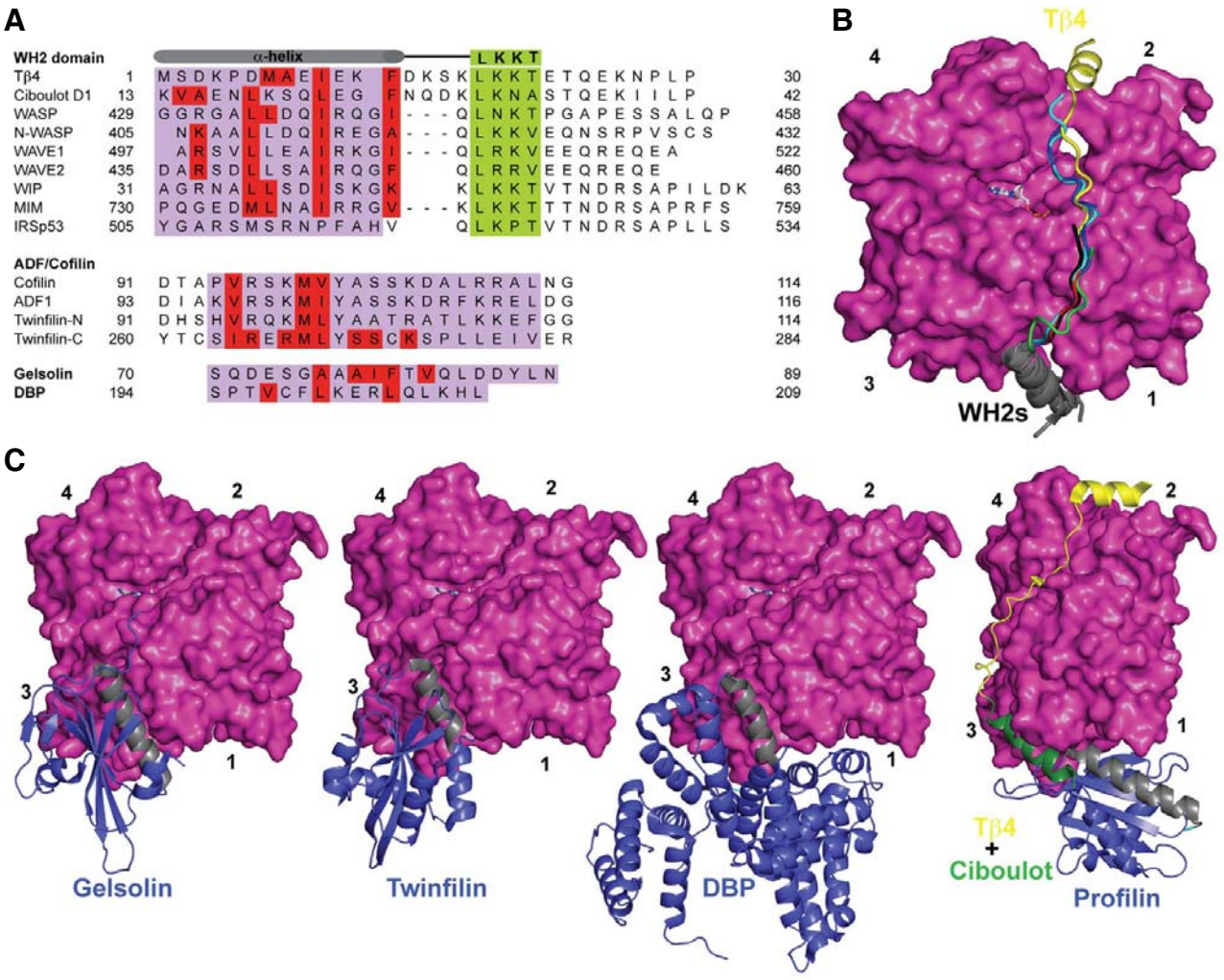

Fig. 4. ABPs frequently present α-helix that binds in the hydrophobic cleft of the actin monomer. (A) Structure-based alignment of the sequences of the helices of ABPs that bind in the hydrophobic cleft of actin. Hydrophobic residues directly implicated in interactions are highlighted in red, and the characteristic "LKKT(V)" motif of the WH2 domain is highlighted in green. (B) Superimposition of the structures of actin complexes with various WH2-containing proteins (ciboulot, green; Tβ4 yellow; MIM, blue; WIP, cyan; WAVE, black; WASP, red). (C) Structures of various ABPs bound to actin, where the helix that binds in the target-binding cleft of actin has been highlighted in grey. Note that profilin binds at the back of the cleft, whereas Tβ4 (whose structure is not known) is related to the WH2 domain, and is therefore expected to bind at the front of the cleft and could bind to actin simultaneously with profilin (Yarmola et al., 2001).

(McGough et al., 2003) and DBP (front to back) (Otterbein et al., 2002), but their main backbones overlap well. Second, actin could be capable of accommodating simultaneously interactions with functionally distinct ABPs. The relatively long helix (four helix turns) of ciboulot spans almost the entire region of the cleft (Hertzog et al., 2004), such that its N-terminal end would partially overlap with profilin, which binds at the back of the cleft (Schutt et al., 1993). In contrast, the shorter helices of gelsolin and most WH2 domains occupy only the front half of the cleft, indicating that the hydrophobic cleft could potentially accept two ABPs simultaneously *in vivo*. Such a co-binding mechanism has been demonstrated at least *in vitro* for profilin and Tβ4 (Fig. 4C) (Yarmola et al., 2007). The common actin-binding pocket also suggests a mechanism of regulation by competition, whereby ABPs compete for binding to the target-binding cleft downstream of signaling pathways, as proposed for the myocardin-related transcription factor (MRTF) (Vartiainen et al., 2007).

The Arp2/3 complex and filament nucleation

Actin filament nucleation and elongation factors that regulate the *de novo* formation of actin filaments in cells have received significant attention lately. Since the identification of the Arp2/3 complex (Machesky et al., 1994), a series of nucleators have been discovered, including formins (Pruyne et al., 2002), Spire (Quinlan et al., 2005), Cobl (Ahuja et al., 2007), VopL (Liverman et al., 2007), VopF (Tam et al., 2007), and Lmod (Chereau et al., 2008), as well as the elongation factors Ena/VASP (Machner et al., 2001). With the exception of formins (Fig. 5C), these molecules all use the WH2 domain for interaction with actin, which in some cases, including Spire, Cobl and VopL/VopF, takes the form of tandem WH2 repeats. In this section, we discuss structural insights into the mechanisms of nucleation of NPFs-Arp2/3 complex. For a more in-depth discussion of other WH2-based filament nucleators the reader can refer to other reviews (Dominguez, 2009; Renault et al., 2008).

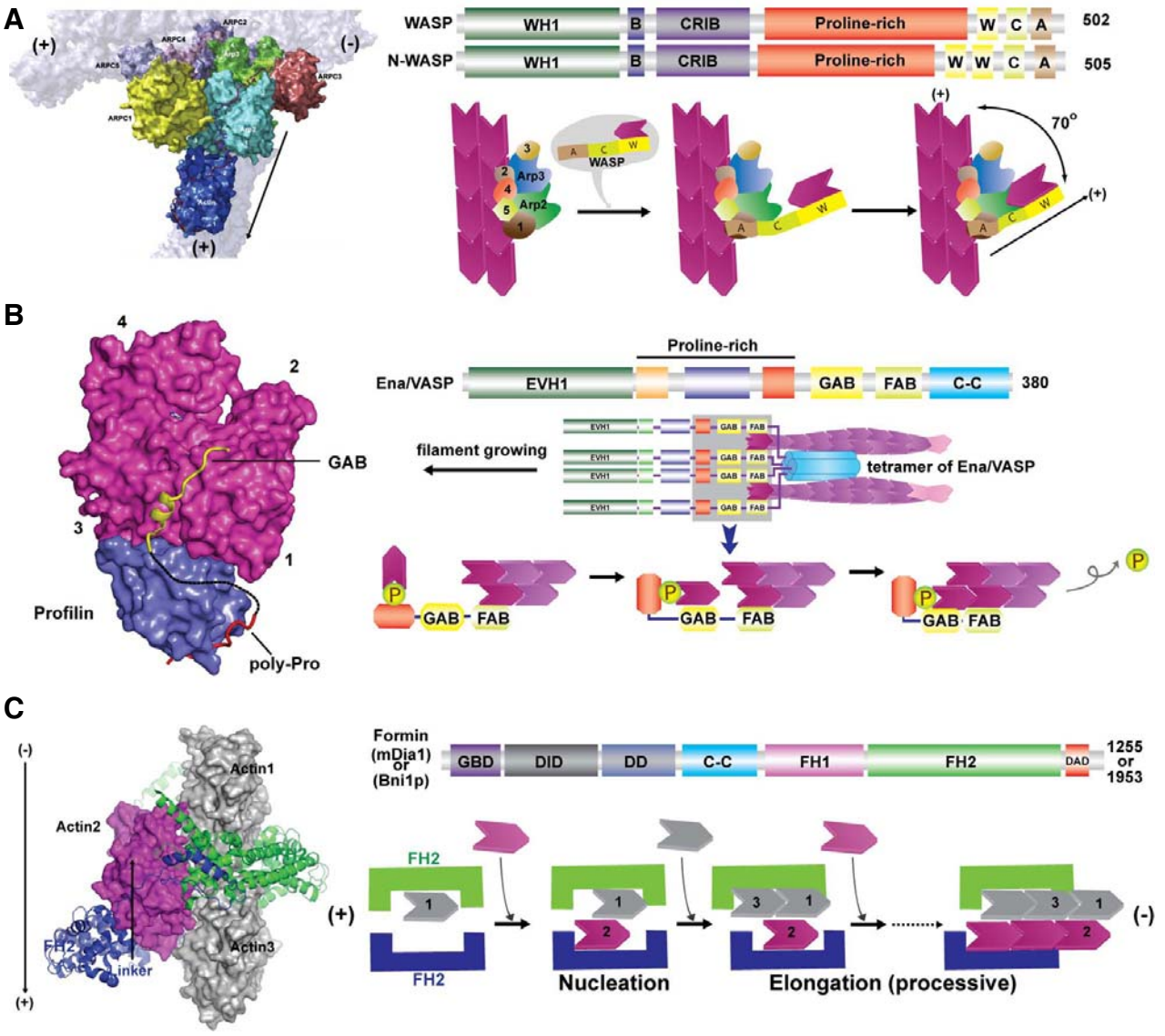

Fig. 5. Proteins involved in actin filament nucleation and elongation. (A) Model of activated Arp2/3 complex, with bound N-WASP WCA and actin, derived from Small Angle X-ray Scattering [adapted from Boczkowska et al. *Structure*. 2008]. By itself, Arp2/3 complex has low nucleation activity, but its activity is increased many folds by Nucleation Promoting Factors (NPFs), such as WASP. Classical NPFs contain a C-terminal C (central) and A (acidic) region that binds Arp2/3 complex and helps stabilize its activated conformation. Immediatelly N-terminal to the C motif is these proteins also contain a WH2 domain that helps recruit the first actin subunit of the new filament that emerges as a branch at a 70° angle from the side of a pre-existing filament. (B) Ena/VASP proteins have a similar domain organization as WASP and N-WASP, although they do not activate the Arp2/3 complex, but instead mediated filament elongation. The G- and F-actin-binding (GAB and FAB) sequences of Ena/VASP are structurally related to the WH2-C region of WASP, whereas the Ena/VASP Homology 1 (EVH1) domain is related to the WASP Homology 1 (WH1) domain of WASP. The structure of the ternary complex of profilin-actin with a poly-Pro-GAB region from VASP confirms this relationship and suggests a model for the transfer of profilin-actin from the cellular pool onto the barbed end of elongating filaments (Ferron et al., 2007). VASP tetramerization, mediated by a C-terminal coiled-coil domain, might be a critical component of its elongation mechanism (Dominguez, 2009). (C) Formin mediated nucleation and elongation. The structure of a complex of formin homology 2 (FH2) domain (blue and green) complexed with actin (Otomo et al., 2005) suggests a mechanism for the stabilization of a short-pitch actin dimer during nucleation and the stair-stepping addition of actin monomers during elongation (Goode and Eck, 2007).

In cells, nucleation, i.e. the formation of small oligomers of two to four actin subunits, is kinetically unfavorable. Moreover, actin monomers are frequently associated with actin-monomer-binding proteins such as profilin and Tβ4, which control their incorporation into filaments. Together, these two factors limit the spontaneous nucleation of actin filaments in cells, thereby creating an opportunity for cells to actively regulate the *de novo* polymerization of actin by using actin filament nucleation and elongation factors. The Arp2/3 complex, which consists of seven proteins including the actin-related proteins Arp2, Arp3 and subunits ARPC1-5, is the most extensively studied of the filament nucleators (Fig. 5A). By itself, the Arp2/3 complex displays very low nucleation activity (Mullins et al., 1998), but it is activated by members of a large family of Nucleation Promoting

Factors (NPFs). Classical NPFs, such as the proteins WASP/WAVE, contain the C-terminal motif WCA (Machesky and Gould, 1999; Machesky et al., 1999). This region consists of three distinct segments: W, C and A. W binds the first actin subunit of the new filament (Figs. 3A and 5A). The C (central or connecting) and A (acidic) motifs interact with various subunits of the Arp2/3 complex, helping to stabilize the activated conformation. The actin monomer bound to the W domain, together with Arp2 and Arp3, are thought to form a trimeric seed for the nucleation of a filament branch that emerges at a 70° angle from the side of a preexisting (mother) filament (Fig. 5A). Thus, in addition to recruiting the first actin subunits (between one and three actin subunits depending on the number of W domains of each specific NPF protein that varies between one and three), NPFs are needed to promote a conformational change within the Arp2/3 complex itself. Indeed, in the inactive structure of the Arp2/3 complex (Robinson et al., 2001), the Arps are separated and their nucleotide-binding clefts are wide open. It is believed that during activation the Arps adopt an actin filament-like conformation. In other words, according to this model (Robinson et al., 2001), Arp2 and Arp3 are the first two subunits at the pointed end of the new filament branch.

Ena/VASP and filament elongation

Ena/VASP family of proteins are WH2-based filament elongation factors, implicated in multiple cellular functions such as axon guidance and the migration of cancer cells (Brindle et al., 1996). These proteins form tetramers through their C-terminal coiled coil (C-C) domain (Bachmann et al., 1999; Kuhnel et al., 2004), and are thought to bind to the barbed ends of actin filament bundles (multiple parallel filaments), catalyzing their synchronized elongation against the plasma membrane (Fig. 2) (Brindle et al., 1996; Krause et al., 2003; 2004). In this way, the filament bundles grow to form cellular structures such as filopodia, which are actin-rich finger-like projections used by cells to sense the environment (Mattila and Lappalainen, 2008).

Like most cytoskeletal proteins, Ena/VASP proteins are modular (Fig. 5B), containing N-terminal Ena/VASP Homology 1 (EVH1), central Pro-rich and C-terminal EVH2 domains (Ferron et al., 2007). The EVH1 domain has a Pleckstrin Homology (PH)-like fold and binds the consensus sequence motif FPPPP in target proteins, helping to localize Ena/VASP proteins to their sites of action. The EVH2 region can be subdivided into G- and F-actin Binding (GAB and FAB) domains, which are both WH2-related sequences, and the C-terminal coiled coil (C-C) tetramerization domain. The central Pro-rich region binds signaling/regulatory proteins and profilin-actin. Such Pro-rich sequences are frequently found among cytoskeletal proteins, including formins and the NPFs of the Arp2/3 complex. The recruitment of multiple profilin-actin complexes to Pro-rich sequences may contribute to increasing the local concentration of actin monomers for polymerization (Kovar et al., 2006).

Structural and biochemical data suggest a molecular model of processive filament elongation by Eva/VASP proteins (Breitsprecher et al., 2008; Chereau and Dominguez, 2006; Ferron et al., 2007; Pasic et al., 2008). According to this model, profilin-actin complexes, the predominant form of polymerization competent actin in cells, are recruited to the Pro-rich region of Ena/VASP and then transferred to the adjacent GAB domain (Fig. 5B). From the GAB domain, the actin monomers can then join the barbed ends of the actin filaments tethered by the FAB domains of the Ena/VASP tetramer. With each monomer addition to the tethered filament barbed ends, Ena/VASP proteins would be expected to step forward (toward the membrane). This is probably accomplished by a mechanism of selective binding, depending on affinity modulation of the GAB and FAB domains respectively to actin monomers and the barbed ends of the actin filaments. Tetramerization is also critical for Ena/VASP function (Bachmann et al., 1999; Kuhnel et al., 2004), presumably by allowing each arm of the Ena/VASP tetramer to release and step forward while the other arms remain bound to the bundle (Ferron et al., 2007). In this way, the Ena/VASP tetramer can processively "track" the barbed end of the actin bundle (Breitsprecher et al., 2008).

Formins

Among filament nucleation/elongation factors, formins mediate the assembly of unbranched actin networks, such as filopodia and stress fibers. The diaphanous-related formins (DRFs), including mDia and Bni1p, are the best studied. Like most cytoskeletal proteins, DRFs are multidomain, multifunctional proteins, comprising GTPase-binding domain (GBD), diaphanous inhibitory domain (DID), dimerization domain (DD), coiled coil (CC), formin homology (FH) 1 and 2 domains, and diaphanous autoregulatory domain (DAD) (Figs. 3B and 5C). These domains play dedicated roles in regulation, dimerization, actin nucleation/elongation and auto-inhibition (Castrillon and Wasserman, 1994; Zigmond, 2004b). In the resting state, formins exist in a folded autoinhibited conformation stabilized by internal DAD-DID interaction (Fig. 3B). The FH2 dimer, responsible for nucleation and elongation, consists of two rod-shaped domains connected in head-to-tail fashion by flexible linkers, whose structural determination immediately suggested that the FH2 dimer "stair-steps" to mediate the sequential incorporation of actin monomers at the barbed-end during processive filament elongation (Xu et al., 2004). Dimerization of the FH2 domain is also necessary for nucleation (Fig. 5C), because each FH2 domain binds an actin monomer such that the ring-like dimer helps stabilize a short-pitch actin dimer (Li and Higgs, 2003; Pruyne et al., 2002). The structure of a formin-actin complex (Otomo et al., 2005) provided further insights into the mechanisms of formin-mediated nucleation and elongation. In the structure, the FH2-actin dimer (built by crystal symmetry) stabilizes a short-pitch actin dimer, which could function as a polymerization nucleus. In addition, this FH2 dimer is in contact with a third actin subunit (labeled actin 3 in Fig. 5C), whose position at the barbed end of the filament is supposedly stabilized by a conformational change leading the uppermost subunit of the FH2 dimer (green subunit in Fig. 5C) to move downwards to bind this newly added actin.

Actin filament crosslinking proteins

Spectrin-family proteins, including α-actinin, spectrin, and dystrophin, are the most important group of proteins involved in crosslinking actin filaments, both among them and with cellular organelles and membranes (Broderick and Winder, 2005). These proteins are predominantly localized to the submembrane cytoskeleton, such as the leading edge and focal contacts of migrating cells (Blanchard et al., 1989; Knight et al., 2000; Otto, 1994; Pascual et al., 1997). Actin crosslinking proteins frequently function as molecular scaffolds, connecting actin filament networks to extracellular matrix proteins, such as ankyrin, laminin and dystroglycan (Figs. 2 and 6B) (Bennett and Baines, 2001; Campbell and Kahl, 1989; Kennedy et al., 1991; Rando, 2001).

Although diverse in size and function, spectrin-family proteins are characterized by the presence of a series of conserved structural modules, including the calponin homology (CH) do-

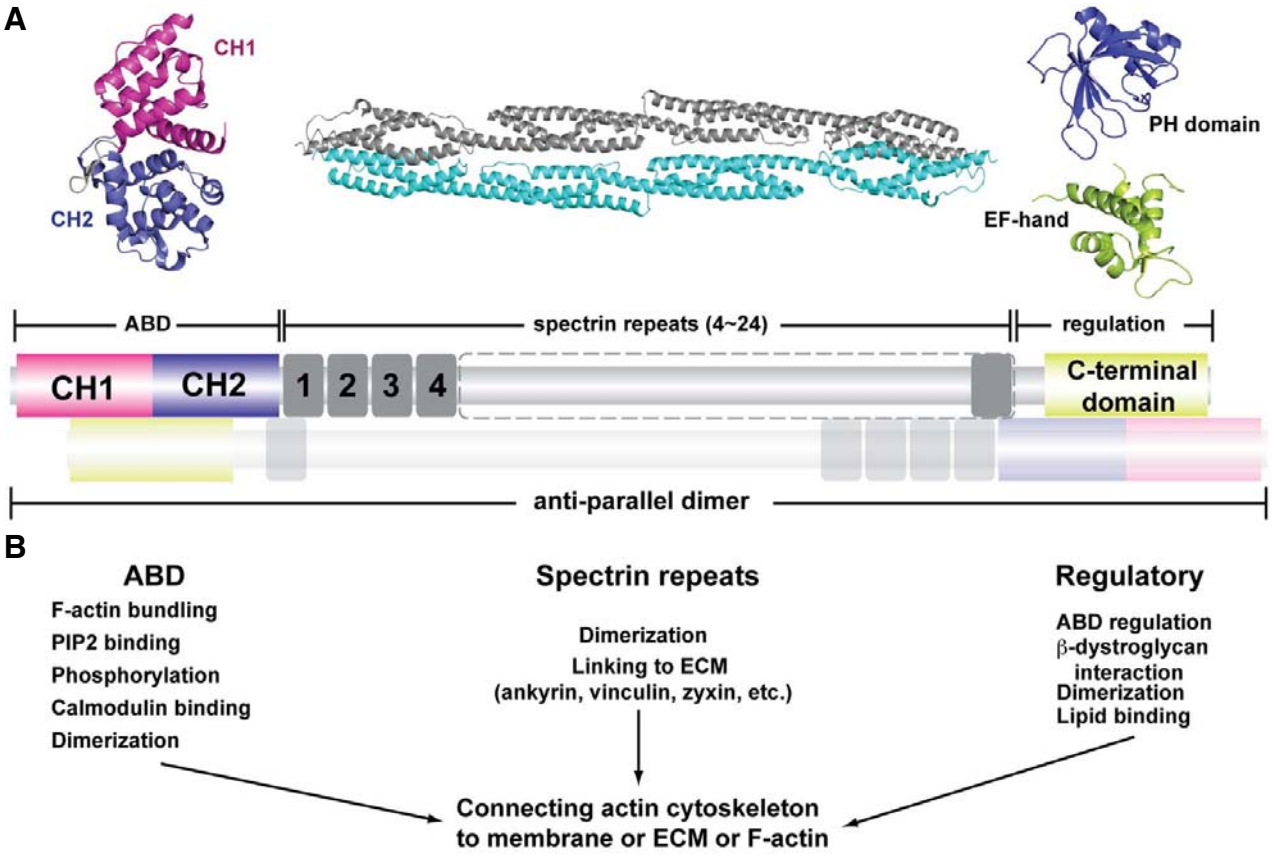

Fig. 6. Spectrin family of actin crosslinking proteins. (A) Domain organization and structures of a prototypical spectrin-family member. Spectrin-family proteins consist of three regions: an N-terminal actin-binding domain (ABD) formed by two calponin homology (CH) domains, a central spectrin repeat region featuring a variable number of spectrin repeats, and a C-terminal domain whose specific fold and function varies from protein to protein. (B) Most (but not all) spectrin-family proteins form anti-parallel dimers (or heterodimers), with the spectrin repeats helping to stabilize tight monomer-monomer interactions. The spectrin repeats also mediate multiple protein-protein interactions (some examples are shown).

main, varying numbers of spectrin repeats, EF-hands, or other regulatory regions (Fig. 6A) (Broderick and Winder, 2005). The CH domain frequently occurs in pairs (CH1 and CH2), which together form the actin-binding domain (ABD) of spectrin-family proteins (Gimona and Winder, 1998).

The crystal structures of several ABDs have been determined, including those of human, A. thaliana and S. pombe fimbrin (Goldsmith et al., 1997; Klein et al., 2004), utrophin (Keep et al., 1999), dystrophin (Norwood et al., 2000), human and mouse plectin (Garcia-Alvarez et al., 2003; Sevcik et al., 2004) and α-actinin 1, 3, 4 (Borrego-Diaz et al., 2006; Franzot et al., 2005; Lee et al., 2008). Generally, these structures display a similar so-called "compact" or "closed" conformation, characterized by extensive interactions between the CH domains. In two of the structures, those of utrophin (Keep et al., 1999) and dystrophin, the compact conformation results from domain swapping between two different molecules in the asymmetric unit (Liu and Eisenberg, 2002). By contrast, the conformation of actin-bound ABDs is debated (Galkin et al., 2002; 2003; Lehman et al., 2004). EM studies of ABD-decorated actin filaments have suggested two different models of binding: compact (Hanein et al., 1998; McGough et al., 1994; Sutherland-Smith et al., 2003) and extended (Galkin et al., 2002; Moores et al., 2000). The compact model holds that the ABD binds actin with only minor changes relative to the crystal structures, whereas the extended model maintains that the two CHs become separated upon binding. It remains to be demonstrated which of these two models is correct.

The spectrin repeats, usually occupy the central region of spectrin-family proteins, and as a result are collectively referred to as the 'rod' domain. The number of spectrin repeats (each repeat consisting of ~110-aa) varies significantly among these proteins, ranging from 4 in α-actinin to ~24 in dystrophin (Broderick and Winder, 2005; Pascual et al., 1997). This provides a mechanism for the regulation of the spacing between actin networks and membrane compartments, which can range from 14 to 130 nm (Fig. 6A) (Amann et al., 1999; Broderick and Winder, 2005; Rybakova et al., 2002; Winder et al., 1995). The spectrin-repeat region also mediates anti-parallel dimerization of various spectrin-family proteins. The structures of various spectrin repeat fragments have been determined, including the entire rod domain of α-actinin (Fig. 6A) (Ylanne et al., 2001a; 2001b).

The C-termini of spectrin-family proteins can vary significantly, and distinct types of domains can occur, including the EF-hand and pleckstrin homology (PH) domains, whose specific nature correlates with the individual functions of each protein (Figs. 6A and 6B). For instance, α-actinin, which functions primarily as an actin filament crosslinking protein, contains two C-terminal EF-hand motifs that are thought to come in close contact with the CH domains within the antiparallel dimer, and contribute to modulating the binding of α-actinin to F-actin in a Ca^{2+}-dependent manner (Blanchard et al., 1989; Lundberg et al.,

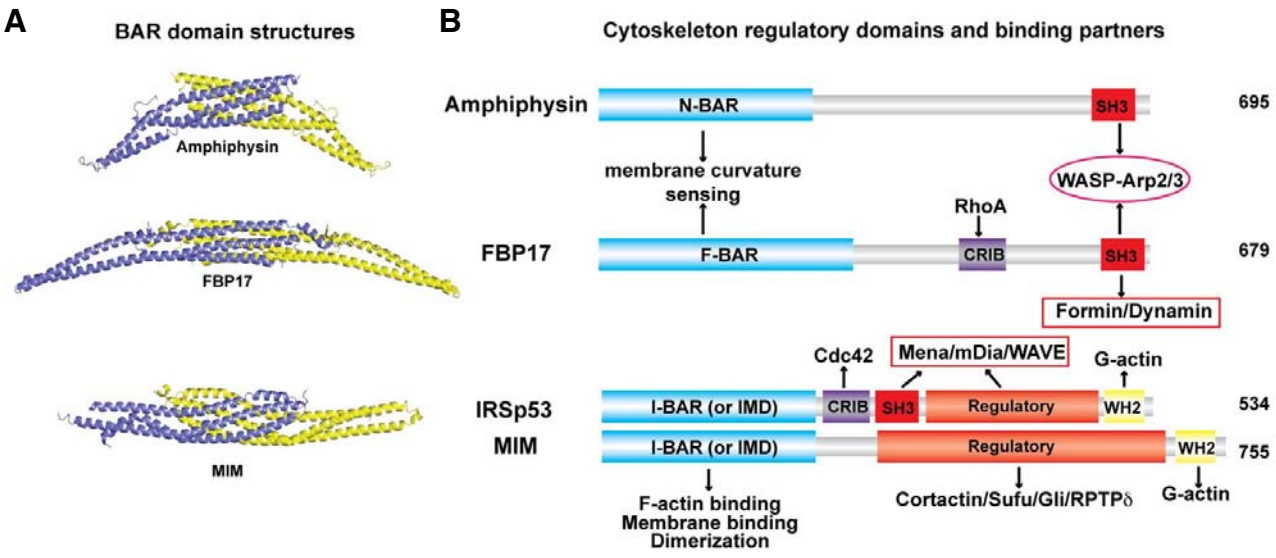

Fig. 7. BAR domain-containing proteins. (A) The BAR domain consists of an elongated anti-parallel dimer of two helical bundles, with a curved, positively charged membrane-binding interface. However, there is significant variation within this fold, and three subclasses are identified based on the overall shape and curvature of the BAR domain: the classical crescent-shaped BAR (found in arfaptin, amphiphysin, and endophilin), the more elongated and less curved F-BAR (found in FBP17, CIP4, FCHo2, and Toca1), and the inverted curvature I-BAR (found in IRSp53, MIM, and ABBA). (B) The BAR domain always occurs in association with other domains, including actin cytoskeleton regulatory, autoinhibitory, and signaling modules. Thus, a number of BAR proteins present PX or PH domains, which provide added affinity for specific membranes and/or become involved in protein-protein interactions. Many BAR proteins also present modules that link to the actin cytoskeleton, such as WW-binding motifs, SH3 and WH2 domains. Also common is the CRIB domain or other motifs that mediate the binding of Rho family GTPases. The types and interactions of these modules, and the specific shape of the BAR domain, determine the function, localization and type of membrane structures (protrusions, invaginations, tubules, vesicles) associated with each BAR domain protein.

1995; Noegel et al., 1987). On the other hand, β-spectrin, which provides a linkage between actin networks and membranes, presents a c-terminal ph domain, which associates with negatively charged phospholipids at the membrane (Ferguson et al., 1995).

BAR proteins linking membrane and actincytoskeleton dynamics

A tight spatial and temporal coordination of actin polymerization and plasma membrane remodeling is a characteristic feature of many cellular processes, including endocytosis, exocytosis, cell motility and intracellular trafficking (Engqvist-Goldstein and Drubin, 2003; Kaksonen et al., 2006; Scita et al., 2008). In these processes, Bin/Amphiphysin/Rvsp (BAR) domain-containing proteins are emerging as key regulators, linking signaling pathways to actin cytoskeleton and membrane dynamics (Fig. 7) (Dawson et al., 2006; Frost et al., 2007; Itoh and De Camilli, 2006; Scita et al., 2008).

BAR proteins are generally unrelated, but are united by a number of features, including the presence of the membrane-binding BAR domain, their modular organization and the fact that they frequently link to the actin cytoskeleton under the control of Rho-family GTPases (Dawson et al., 2006; Frost et al., 2008; Henne et al., 2007; Itoh and De Camilli, 2006; Scita et al., 2008). Because of the lack of overall sequence similarity, the BAR family has expanded with the determination of crystal structures of the dimerization/membrane-binding domains of proteins originally thought to be unrelated (Habermann, 2004; Millard et al., 2005; Peter et al., 2004; Shimada et al., 2007). The BAR fold consists of an elongated anti-parallel dimer of two helical bundles, with a curved, positively charged membrane-binding interface (Fig. 7A) (Govind et al., 2001; Gallop et al., 2006; Henne et al., 2007; Lee et al., 2007; Masuda et al., 2006; Millard et al., 2005; Peter et al., 2004; Pylypenko et al., 2007; Shimada et al., 2007; Tarricone et al., 2001; Weissenhorn, 2005; Zhu et al., 2007). However, there is significant variation within this fold, and three subfamilies are identified based on the overall shape and curvature of the BAR domain (Frost et al., 2007; Henne et al., 2007; Scita et al., 2008): the classical crescent-shaped N-BAR (found in arfaptin, amphiphysin, and endophilin), the more elongated and less curved F-BAR (found in FBP17, CIP4, FCHo2, and Toca1), and the inverted curvature I-BAR (found in IRSp53, MIM, and ABBA) (Fig. 7A). Some BAR domains present helical appendages (N-BAR) that are thought to penetrate the bound membrane (Gallop et al., 2006; Saarikangas et al., 2009). There is a direct correlation between the shape and size of each BAR domain and the shape and curvature of the membranes associated with them (Fig. 7A) (Frost et al., 2008; Mattila et al., 2007; Peter et al., 2004; Saarikangas et al., 2009; Shimada et al., 2007).

The question of membrane curvature sensing versus generation has received significant attention. While the original view was that BAR proteins primarily 'senseor' or 'stabilize' curvature (Peter et al., 2004), recent electron microscopy evidence suggests that at least some BAR domains can bind flat membranes and induce curvature *via* cooperative BAR-BAR interactions that are more favorable on membranes (Frost et al., 2008). This latter study also showed that membrane tubules form when F-BARs polymerize into helical coats that are held together by lateral and tip-to-tip BAR-BAR interactions, as well as interactions of the BAR domain with the membrane itself. Similar interaction may occur with the I-BAR domain, but what distinguished the I-BAR domain is that it induces membrane curvature in the opposite direction to that of the N-BAR and F-

vature in the opposite direction to that of the N-BAR and F-BAR domains, and bind to the interior of membrane compart-ments (Fig. 2) as supposed to the exterior for the N-BAR and F-BAR domains (Mattila et al., 2007; Saarikangas et al., 2009; Suetsugu et al., 2006). The formation of endocytic vesicles is generally driven by BAR proteins that sense membrane curvature and/or actively bend the membrane. Proteins such as Arfarptin and Tuba, containing crescent-shaped membrane-binding BAR domains, sense the curvature of clathrin-coated vesicle, which are ~200 Å in diameter (Salazar et al., 2003; Tarricone et al., 2001). Meanwhile, the N-BAR domains of amphiphysin and endophilin are involved in the formation of the narrowly curved necks of nascent vesicles. These endocytic vesicles are ultimately pinched-off from the membrane by dynamin, a GTPase that catalyzes vesicle scission from the plasma membrane (Fig. 7B) (Gallop et al., 2006; Shafer and Voss, 2004). F-BAR proteins, such as FBP17, syndapin and Toca1, are also implicated in clathrin-mediated endocytosis in cooperation with dynamin (Ho et al., 2004; Kamioka et al., 2004; Kessels and Qualmann, 2004).

The coordination between BAR domain proteins and the actin cytoskeleton is just beginning to be unraveled, but it appears to affect most cellular processes of the actin cytoskeleton. Despite the extraordinary plasticity of the BAR domain, what sets BAR proteins apart is their functional diversity, resulting from the presence of additional modules N- and C-terminal to the BAR domain (Fig. 7B). Thus, a number of BAR proteins present PX or PH domains, which provide added affinity for phospholipid membranes and/or become involved in protein-protein interactions (Pylypenko et al., 2007; Zhu et al., 2007). Many BAR proteins also present modules that link to the actin cytoskeleton, such as WW-binding motifs, SH3 and WH2 domains (Scita et al., 2008; Shimada et al., 2007). Also common is the CRIB domain or other motifs that mediate the binding of Rho GTPases. Most BAR proteins appear to exist in an auto-inhibited conformation, a widespread feature among cytoskeletal proteins (Fig. 3C). Thus, a conformational change in IRSp53 resulting from the binding of GTPases to its CRIB motif is thought to free the SH3 domain to recruit ABPs such as WASP/Scar that in turn engage in actin cytoskeleton remodeling (Figs. 3C and 7B) (Govind et al., 2001; Miki et al., 2000; Nakagawa et al., 2003; Takenawa and Suetsugu, 2007). During filopodia and lamellipodia formation, I-BAR proteins are recruited to the membrane and subsequently could direct the barbed ends of the actin filaments toward the plasma membrane by directly interacting with ABPs in response to extracellular stimuli (Ahmed et al., 2009).

SUMMARY AND PERSPECTIVES

The dynamic remodeling of the actin cytoskeleton is an essential component of many cellular processes, including cell locomotion, cytokinesis and membrane trafficking. Additionally, many pathogens hijack the host cell actin cytoskeleton during infection. These processes involve rapid bursts of actin polymerization/depolymerization with remarkable spatiotemporal precision. Actin and a myriad of actin-binding proteins become involved in the regulation of these processes. A wide range of diseases, including cancer, neurological and musculoskeletal disorders result from malfunctioning of cytoskeletal proteins. While the study of actin cytoskeleton dynamics has recently intensified, what is critically lacking is a comprehensive structure-function understanding of the interplay between the many membrane binding, scaffolding and signaling proteins that conform the cytoskeleton. These questions are likely to dominate the research within the actin cytoskeleton field during the following few years.

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