Formins: signaling effectors for assembly and polarization of actin filaments

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Summary

Eukaryotic cells require filamentous actin to maintain their shape and for movement, growth and replication. New actin filaments are formed by the cutting of existing filaments or de novo through the action of specialized nucleators. The most highly characterized nucleator is the Arp2/3 complex, which nucleates the branched actin networks in the lamellae of migrating cells. Recently, Bni1p, which is a member of the formin family of proteins, has been shown to nucleate actin filaments in vitro. Formins are implicated in the formation of actin cables in yeast, stress fibers in tissue culture cells and cytokinesis in many cell types. Formins contain two highly conserved formin-homology domains, FH1 and FH2. The Bni1p FH2 domain is sufficient to mediate nucleation. The Bni1p FH1 domain binds profilin, an actin-monomer-binding protein that delivers actin to the growing barbed end of filaments. The Bni1p FH1-profilin interaction enhances nucleation. Formins participate in a number of signaling pathways that control the assembly of specific actin structures and bind the barbed end of actin filaments, thereby providing a cytoskeletal basis for the establishment of cell polarity.

Key words: Formins, Bni1p, Cell polarity, Actin assembly, Signal transduction

Introduction

Actin filaments contribute to many processes in eukaryotic cells, including polarization, protrusion, contraction, nuclear segregation, cytokinesis and vesicle trafficking. Each of these processes involves different actin-binding proteins and different arrangements of actin filaments.

Actin filaments are polar, having a fast-growing 'barbed' end and a slow-growing 'pointed' end. Actin elongation in cells occurs largely, if not exclusively, at the barbed end. Indeed, proteins and small molecules, such as capping protein and cytochalasin, that cap filament barbed ends (Caldwell et al., 1989; Tellam and Frieden, 1982) block actin assembly in vivo. Thus, cells can promote actin assembly by uncapping a filament's barbed end, by severing a filament to create a new barbed end or by de novo nucleation of an actin filament.

For a number of filamentous actin structures, nucleation depends on activation of the Arp2/3 complex. For example, Arp2/3 nucleates branched networks that contain short actin filaments found in the lamellipodia of motile cells (reviewed in Higgs and Pollard, 2001; Pollard et al., 2000; Pollard et al., 2001). The actin branches form at 70° angles and are thought to be optimized for mediating protrusion (Fig. 1). The Arp2/3 complex is also a critical component of yeast cortical actin patches, which appear to control endocytosis (reviewed in Pruyne and Bretscher, 2000), and is recruited by pathogenic bacteria (such as *Listeria monocytogenes*, *Shigella flexneri*, *Salmonella typhimurium* and enteropathogenic *Escherichia coli*) and viruses (such as *Vaccinia*), which rely on the host's actin polymerization for movement (reviewed in Higgs and Pollard, 2001).

Genetic evidence suggests that the Arp2/3 complex is not the nucleator of all actin filaments. In budding yeast, its deletion disrupts the assembly of cortical actin patches but does not affect the assembly of actin cables or the actin contractile ring (Evangelista et al., 2002; Tolliday et al., 2002; Winter et al., 1999). In *Drosophila melanogaster*, mutations in Arp2/3 or its activators do not affect cytokinetic contractile rings, the actin-dependent cytoplasmic bridges and ring canals that appear in nurse cells early in development, the ordered actin bundles that form in nurse cells late in development or actin bundles in bristles (Fig. 1) (reviewed in Miller, 2002; Hudson and Cooley, 2002). Furthermore, reduced levels of Arp2 in *Caenorhabditis elegans* do not prevent cytokinesis or assembly of the cortical microfilaments needed to establish an anterior-posterior axis (Severson et al., 2002). Here, we review recent findings suggesting that nucleation of at least some of these Arp2/3-independent structures is controlled by members of the formin family of proteins.

Formins: multidomain proteins controling actin assembly

Formins are a family of highly conserved eukaryotic proteins implicated in a wide range of actin-based processes, including cell polarization, cytokinesis, hair cell stereocilia formation, sperm cell acrosome formation and embryonic developmental (e.g. limb and kidney morphogenesis) (see Table 1) (reviewed in Ridley, 1999; Wasserman, 1998; Zeller et al., 1999). The mouse *limb deformity* (*ld*) gene encodes the first formin

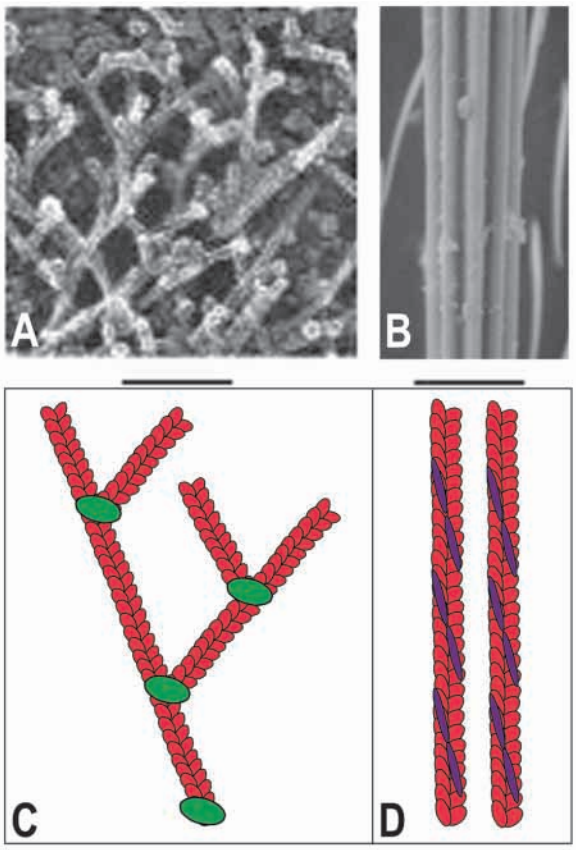

Fig. 1. The branched actin networks found in fibroblast lamellipodia (A) and actin bundles in a bristle of *Drosophila melanogaster* (B) [Adapted with permission (Hudson and Cooley, 2002; Svitkina and Borisy, 1999)] (bar, 0.1 μm); a schematic diagram showing branched (C) and unbranched (D) actin filaments. The Arp2/3 complex (green) crosslinks and stabilizes branched filaments, whereas tropomyosin (purple) stabilizes unbranched filaments.

identified – so named because mice with mutant alleles fail to 'form' proper limbs and kidneys (Kleinebrecht et al., 1982; Mass et al., 1990; Woychik et al., 1985; Zeller et al., 1989).

The most highly conserved feature of formins is the two juxtaposed formin homology domains, FH1 and FH2 (Castrillon and Wasserman, 1994; Wasserman, 1998), both of which are implicated in control of actin assembly (Evangelista et al., 1997; Evangelista et al., 2002; Sagot et al., 2002a; Watanabe et al., 1999) (Fig. 2A). Some formins also contain a conserved FH3 motif(s) between the RBD and the FH1 domain (Fig. 2A); this motif determines subcellular localization (Petersen et al., 1998; Kato et al., 2001; Oazki-Kuroda et al., 2001; Sharpless and Harris, 2002). The conservation of the formin homology domains suggests that all formins have similar molecular activities and underscores the need to characterize their structure and function.

The proline-rich FH1 domain binds to the G-actin-binding protein profilin (Chang et al., 1997; Evangelista et al., 1997; Imamura et al., 1997; Watanabe et al., 1997). The formin-profilin interaction, which can deliver ATP-bound actin monomers to the growing barbed ends of actin filaments (reviewed in Carlier and Pantaloni, 1997; Theriot and Mitchison, 1993; Wear et al., 2000), is essential for at least some formin functions (see below). SH3 and WW domains (Chan et al., 1996; Kamei et al., 1998; Tong et al., 2002; Vallen et al., 2000) also associate with the FH1 domain of formins. For example, the SH3 domain of yeast Hof1p, a protein that participates in cytokinesis, binds to the FH1 domain of Bnr1p in a Rho-dependent manner (Kamei et al., 1998; Vallen et al., 2000). Similarly, the SH3 domain of the mammalian nonreceptor tyrosine kinase Src binds to mammalian diaphanous (mDia)-related formins (DRFs; see below) (Tominaga et al., 2000). The mDia-Src interaction appears to mediate mDia-dependent signaling to serum response factor (SRF), a transcription factor that regulates growth-factor-induced genes and muscle-specific genes (Tominaga et al.,

Table 1. Annotated formins and their proposed functions

Protein	Organism	Proposed role
DFNA1*	Human	Stereocilia formation (Lynch et al., 1997)
hDIA*	Human	Acrosome formation (Zhang, 2001)
Daam1	Human	Signaling (Habas et al., 2001)
FHOS*	Human	Spleen morphogenesis (Westendorf, 2001; Westendorf et al., 1999)
Formin-1	Mouse	Cell signaling, limb and kidney morphogenesis (Kleinerbrecht et al., 1982; Maas et al., 1990; Woychik et al., 1985; Zeller et al., 1989)
Formin-2	Mouse	Neural function and oogenesis (Leader and Leder, 2000; Leader et al., 2002)
Delphilin	Mouse	Cell signaling (Miyagi, 2002)
mDia1*	Mouse	Stress fiber formation, actin assembly, activation of SRF (Copeland and Treisman, 2002; Geneste et al., 2002; Tominaga et al., 2000; Watanabe et al., 1999)
mDia2*	Mouse	Stress fiber formation, activation of SRF (Tominaga et al., 2000)
Diaphanous*	*Drosophila*	Cytokinesis (Castrillon and Wasserman, 1994)
Cappuccino	*Drosophila*	Cell polarity (Emmons et al., 1995; Manseau and Schupbach, 1989)
CYK-1	*C. elegans*	Cytokinesis (Swan et al., 1998; Severson et al., 2002)
AFH1	*A. thalania*	Cell polarity (Banno and Chua, 2000)
SepA*	*A. nidulans*	Cell polarity, cytokinesis (Harris et al., 1997; Sharpless and Harris, 2002)
Bni1p*	*S. cerevisiae*	Cell polarity, cytokinesis, actin cable formation, actin nucleation (Evangelista et al., 1997; Evangelista et al., 2002; Imamura et al., 1997; Sagot et al., 2002a; Pruyne et al., 2002; Sagot et al., 2002b; Zahner et al., 1996)
Bnr1p*	*S. cerevisiae*	Cytokinesis (Imamura et al., 1997; Kamei et al., 1998; Kikyo et al., 1999; Vallen et al., 2000)
For3	*S. pombe*	Cell polarity, actin cable formation (Feierbach and Chang, 2001)
Cdc12	*S. pombe*	Cytokinesis (Arai and Mabuchi, 2002; Chang et al., 1997; Pelham and Chang, 2002; Streiblova et al., 1984)
Fus1	*S. pombe*	Cell polarity (Petersen et al., 1998; Petersen et al., 1995)

*Diaphanous-related formins.

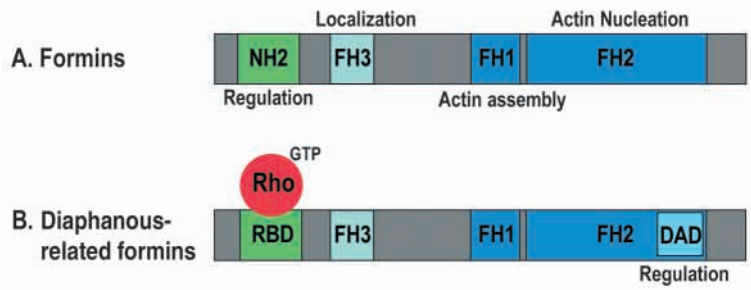

Fig. 2. (A) Domain organization of the formin family of proteins. Shown is the formin-homology 1 (FH1) domain, the FH2 domain, which controls actin nucleation, and the FH3 domain, which is important for localization of some formins. (B) Domain organization of the Diaphanous-related formins (DRFs). Unique to this class of proteins is the regulatory Rho-binding domain (RBD), which binds activated forms of Rho GTPases and the Dia-autoregulatory-domain (DAD), which mediates an intramolecular auto-inhibitory interaction with RBD. An activated formin, with the DAD-RBD intramolecular interaction relieved by Rho-GTP binding, is shown.

2000). Further analysis (Copeland and Treisman, 2002) has revealed that fragments of mDia1 containing only the FH2 domain stimulate actin assembly by causing a decrease in the pool of G-actin, which leads to activation of SRF indirectly. Thus, the FH1 domain appears to function as a scaffold that enables formins to recruit proteins that modulate its intrinsic actin assembly activity.

The FH2 domain represents a unique and defining feature of formin proteins, sharing no obvious sequence similarity with any other domain or polypeptide. It controls actin nucleation in vitro (Pruyne et al., 2002; Sagot et al., 2002b) and actin assembly in vivo (Copeland and Treisman, 2002; Evangelista et al., 2002; Pruyne et al., 2002; Sagot et al., 2002a; Sagot et al., 2002b). The domain was originally thought to encompass 100 residues (Castrillon and Wasserman, 1994; Wasserman, 1998), but sequence alignment (http://pfam.wustl.edu/index.html) of multiple newly discovered formins indicates that the region of sequence similarity is much larger and covers almost 500 residues, which encompass the C-terminal Dia-autoregulatory (DAD) domain (see below). The FH2 domain remains to be fully characterized, so we do not yet know whether it is composed of multiple subdomains that have independent actin assembly roles. In the case of mDia1, deletion analysis has demonstrated that the N-terminal and C-terminal regions of the FH2 domain are both required for efficient actin assembly in vivo (Copeland and Treisman, 2002). Consistent with this observation is the finding that deletion of the C-terminal region of Bni1p encompassing the DAD domain results in a 50% loss of activity (M.E. and C.B., unpublished). Thus most of the FH2 may be dedicated to an actin assembly role.

Some evidence, nevertheless, suggests that particular subdomains of the FH2 domain may have distinct actin assembly functions. For example, the N-terminal region of the Bni1p FH2 domain binds translation elongation factor 1A (eF1A) (Umikawa et al., 1998; Liu et al., 2002). Since eF1A can bundle actin filaments (Demma et al., 1990), this interaction may facilitate the organization of growing filaments or regulate actin assembly in some other fashion. Overexpression of eF1A in yeast induces filament assembly resembling that associated with activated forms of Bni1p (Munshi et al., 2001). Deletion of the eF1A-binding site within Bni1p leads to a defect in actin assembly in vivo (Umikawa et al., 1998); however, to determine whether this effect is due to a defect in eF1A interaction or in nucleation, the mutant protein should be assessed for actin nucleation activity in vitro (discussed below).

A distinct class of formins, the DRFs (Table 1, asterisk), has been defined on the basis of their ability to interact with the activated GTP-bound form of a Rho-type GTPase through an N-terminal Rho-binding domain (RBD) (Evangelista et al., 1997; Habas et al., 2001; Imamura et al., 1997; Kohno et al., 1996; Watanabe et al., 1997) (Fig. 2B). Because the formin RBD domains do not share significant sequence similarity, DRFs have been classified by a functional definition. Rho-GTP binding to the RBD domain alleviates auto-inhibition by a DAD domain (Fig. 2B), which mediates an intramolecular interaction with the RBD, producing an inactivated state (Alberts, 2001; Watanabe et al., 1999). This regulatory activity was originally characterized in mDia1 (Watanabe et al., 1999), and a consensus DAD domain is a general feature of DRFs (Alberts, 2001). This type of regulation resembles that associated with Cdc42-GTP-dependent activation of the actin regulator N-WASP (Rohatgi et al., 1999). Because a number of formins whose activation has not been characterized contain sequences resembling the DAD domain (e.g. *S. pombe* For3), they may also be DRFs.

Some formins may be regulated by other signaling pathways or different mechanisms. If these other formins retain an intramolecular mode of regulation, then any protein that binds or modifies either the N-terminal regulatory region or the corresponding DAD-like negative regulatory domain could potentially activate nucleation. For example, mammalian Delphilin links the glutamate receptor δ2 (GluRδ), involved in postsynaptic transmission, to the actin cytoskeleton (Miyagi et al., 2002). Delphilin is a unique formin because it contains an N-terminal PDZ domain, which binds GluRδ2 (Miyagi et al., 2002). In this case, the GluRδ2 receptor may also regulate the activity of Delphilin in a manner analagous to Rho-GTP-mediated regulation of DRFs. In response to Wnt-activated Frizzled (Fz) receptor signaling, the human formin Daam1 forms a complex with Dishevelled (Dvl) and activated RhoA (Habas et al., 2001). Finally, the plant formin AHF1 contains an N-terminal transmembrane domain, which has the potential to localize or regulate its function (Banno and Chua, 2000).

Formins directly assemble Arp2/3-independent actin structures

The first evidence that formins catalyze the assembly of filamentous actin structures was obtained from yeast cells overexpressing N-terminal truncations of Bni1p, which show increased levels of filamentous actin cables (Evangelista et al., 1997). Subsequently, truncations of mammalian mDia1 were shown to cause an increase in actin stress fiber formation (Watanabe et al., 1999) and increase the cellular F-actin content (Copeland and Treisman, 2002).

More recent work has established a direct role for budding yeast formins (Bni1p and Bnr1p) in the assembly of actin cables (Evangelista et al., 2002; Pruyne et al., 2002; Sagot et al., 2002a; Sagot et al., 2002b). Deletion of *BNI1* or *BNR1* has no effect on cell viability, but deletion of both genes is lethal (Imamura et al., 1997), indicating that the roles of yeast formins overlap. Creation of fast-acting, temperature-sensitive (ts) mutations in the FH2 domain of *BNI1* in a *bnr1Δ* background has allowed investigation of the roles of these formins in vivo (Evangelista et al., 2002; Sagot et al., 2002a). At permissive temperature, *bnr1Δ bni1-ts* double mutants show wild-type actin cables and polarized cortical patches (Fig. 3). After a shift to the restrictive temperature, the actin cables disappear rapidly, but there is no change in the structure or polarization of actin patches (Fig. 3). Similarly, in fission yeast, the formin For3 appears to play a major role in actin cable formation (Feierbach and Chang, 2001). Analysis of an *arp3Δ* deletion mutant revealed that it exhibits defective actin patch assembly but normal actin cable assembly (Winter et al., 1999; Evangelista et al., 2002). Thus, in yeast, Arp2/3 appears to be dedicated to actin patch assembly whereas formins are dedicated to actin cable assembly.

In *C. elegans* (Severson et al., 2002), RNAi-induced reduction in the levels of either profilin or the formin CYK-1 results in cytokinesis and anterior-posterior polarity defects. In contrast, the Arp2/3 complex is dispensable for both of these processes but is required for other actin-based processes such as gastrulation and epidermal enclosure. Thus, in *C. elegans*, the Arp2/3 complex and formins might also assemble distinct actin structures.

Formin FH2 domain: nucleation and polarization of actin filaments

If formins assemble actin filaments independently of Arp2/3, how are formin-induced filaments generated? The simplest possibility is that formins nucleate actin filaments directly. In fact, C-terminal fragments of Bni1p expressed and purified from *E. coli* stimulate actin nucleation in vitro (Pruyne et al., 2002; Sagot et al., 2002b), and deletion analysis has revealed that the Bni1p FH2 domain is sufficient for actin filament nucleation (Pruyne et al., 2002).

Polymerization induced by Bni1p is inhibited by cytochalasin B, which indicates that the nucleated filaments grow predominantly from the fast-growing barbed end (Pruyne et al., 2002; Sagot et al., 2002b). Electron microscopy has shown that these filaments are long and unbranched (Pruyne et al., 2002; Sagot et al., 2002b) and that Bni1p associates specifically with the barbed end (Pruyne et al., 2002). Indeed, a Bni1p construct containing just the FH1 and FH2 domains (Bn1p FH1-FH2) can associate with the barbed end of pre-existing filaments and slow but not block their elongation (Pruyne et al., 2002). These findings suggest that Bni1p is an actin-filament-capping protein, but, unlike traditional capping proteins, which bind to the barbed end and prevent filament elongation, Bni1p appears to bind to the barbed end of filaments and regulate the rate at which they grow.

Since Bni1p localizes to discrete regions of the growing cell cortex, such as the tip of a developing bud, its ability to bind to the barbed ends of filaments indicates that it may be able to tether the filaments it nucleates and polarize them towards itself

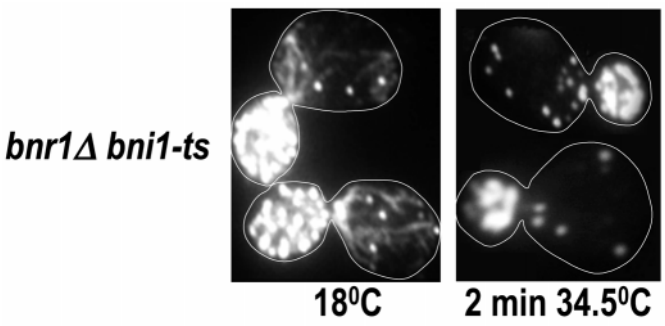

Fig. 3. Yeast formin proteins Bni1p and Bnr1p are specifically required for the assembly of actin cables but not actin patches. *bnr1Δ bni1-ts* (temperature-sensitive) mutants show normal actin cables at permissive temperatures (18°C) but rapidly lose cables at restrictive temperatures (34.5°C) with no effect on cortical actin patches.

(Fig. 4). Indeed, the unidirectional movement of cable-dependent myosin V motors indicates that cable filaments are polarized with their barbed ends towards the growing cortex (Schott et al., 2002). In vivo analysis of cable dynamics has shown that actin cables assemble from these sites, indicating that the anchorage of actin cables occurs at their growing ends (Yang and Pon, 2002). Because myosin V cargo includes secretory vesicles, mRNA and a molecular complex that associates with cytoplasmic microtubules (Beach et al., 1999; Bertrand et al., 1998; Hoepfner et al., 2001; Miller et al., 2000; Pruyne et al., 1998; Rossanese et al., 2001; Schott et al., 2002; Takizawa et al., 2000; Theesfeld et al., 1999; Yin et al., 2000; Zahner et al., 1996) (Fig. 4), mutations in the *BNI1* gene lead to defects in polarized secretion, mRNA localization, spindle orientation and nuclear positioning (Evangelista et al., 2002; Fujiwara et al., 1999; Lee et al., 1999; Sagot et al., 2002a). Thus, the ability of Bni1p, possibly aided by other proteins, to bind to the growing barbed end of an actin cable filament provides a simple yet elegant way of orienting actin cables, which directs myosin motors and their associated cargo towards the growing bud tip (Fig. 4).

Mechanism for FH2-mediated actin nucleation

Modeling of the Bni1p-mediated in vitro assembly reaction (Pring et al., 2003) suggests that the FH2 domain stabilizes an actin dimer and that this complex functions as a nucleation unit that enables efficient barbed end growth (Fig. 5A). At low G-actin concentrations, the spontaneous formation of actin dimers is rare because of their instability; however, formins could create a dimer by binding actin monomers sequentially. In this case, the nucleation efficiency would depend upon the affinity of the FH2 domain for G-actin. Preliminary analysis indicates that Bni1p FH1-FH2 has relatively weak binding affinity for G-actin (i.e. >5 μm) (S.Z., unpublished), which is consistent with its modest nucleating activity.

It is interesting to compare features of spontaneous and Arp2/3-induced actin nucleation with that induced by formins. In spontaneous nucleation, the pre-nucleus (a form that can elongate like an actin filament) is an actin trimer, which forms very slowly at physiological actin concentrations. In Arp2/3-induced nucleation, the actin-related proteins Arp2 and Arp3 are thought to acquire an arrangement similar to that of two

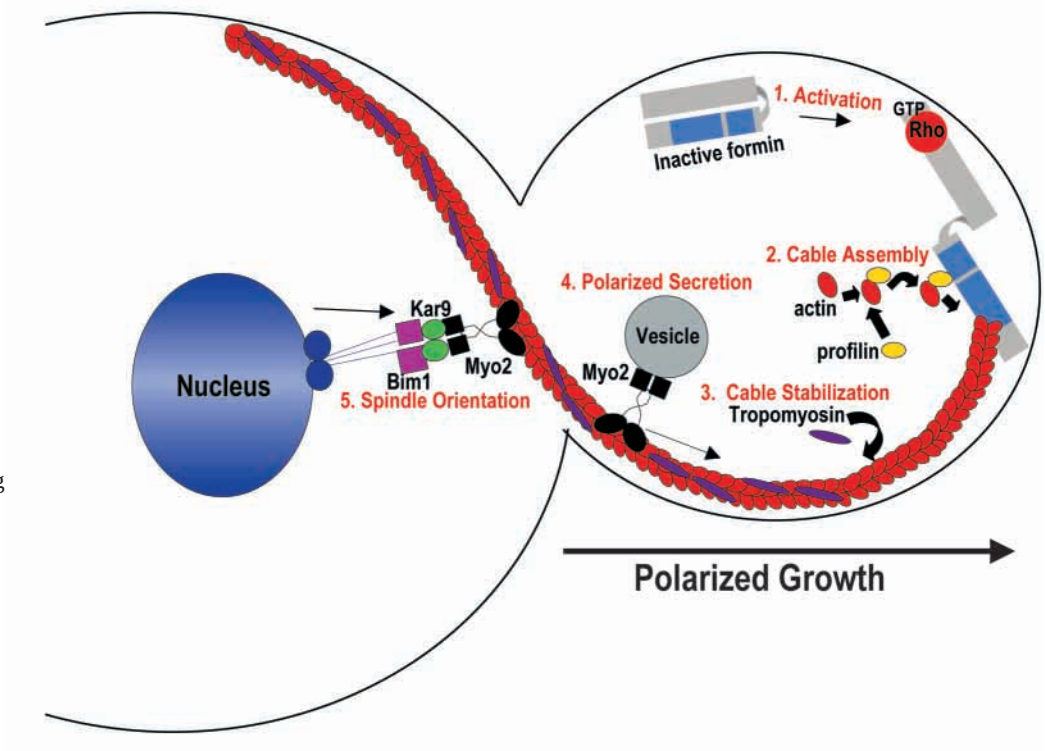

Fig. 4. Bni1p controls cell polarity in budding yeast through the assembly of actin cables. Bni1p is activated by Rho GTPases (1). Activated Bni1p nucleates and assembles actin cables (2). Tropomyosin stabilizes the growing cables (3). Bni1p binds to the barbed ends of cables, thereby establishing their polarity. In turn, the polarized cables can then serve as tracks for myosin V, Myo2p, which migrates towards the barbed end of the actin cable to deliver secretory vesicles (4) and to orient the mitotic spindle (5).

actin subunits (along the short-pitch helix) of an actin filament (reviewed in Pollard and Beltzer, 2002). Addition of a single G-actin molecule to the Arp2/3 complex forms a nucleus that can elongate with kinetics of an actin filament barbed end (Fig. 5A); the resultant filament is capped by the Arp2/3 complex at its pointed end (Fig. 5B).

In Bni1p FH2-induced nucleation, the pre-nucleus is an actin dimer (Fig. 5A), which is probably formed by sequential monomer addition. Like capping protein and cytochalasin, Bni1p also associates with the barbed end of the filament (Fig. 5B); however, it slows but does not block barbed end elongation, acting like a 'leaky capper'. The underlying mechanism remains a mystery, but perhaps the FH2 domain wobbles towards the barbed end in a processive manner as the filament is elongating.

Arp2/3-dependent nucleation is regulated by various factors, including WASP family members and cortactin (reviewed in Higgs and Pollard, 2001). As noted above, at least some formins are activated by Rho GTPases. Formin-induced nucleation may be further modulated by post-translational modification of formin or its binding partners. For example, mouse formin I (Vogt et al., 1993) and yeast Bni1p (Goehring et al., 2003), are phosphoproteins in vivo and could be regulated by phosphorylation. Formin-interacting proteins that might participate in the assembly reaction include Bud6p/Aip3p (Amberg et al., 1997), a protein that is required for Bni1p-induced cable formation in vivo (Evangelista et al., 2002; Sagot et al., 2002a) and in two-hybrid studies interacts with a Bni1p C-terminal region that overlaps with the FH2 domain (Evangelista et al., 1997), and the actin-bundling protein eF1A (Demma et al., 1990), which interacts with the N-terminal region of the FH2 domain (Umikawa et al., 1998; Liu et al., 2002). We anticipate that in vitro actin assembly reactions with full-length formin proteins purified from eukaryotic cells and formin-binding proteins will provide further insight into the regulation of formin-induced actin assembly.

The role of profilin in Bni1p-induced nucleation

In mammalian cells, the FH2 domain of mDia is sufficient to induce accumulation of filamentous actin (Copeland and Triesman, 2002). In budding yeast, the Bni1p FH1 domain and profilin, an actin-monomer-binding protein, are required in addition to the FH2 domain to assemble actin filaments in vivo (Evangelista et al., 2002; Pruyne et al., 2002; Sagot et al., 2002a; Sagot et al., 2002b). In vitro, profilin enhances nucleation by Bni1p FH1-FH2 (Pring et al., 2003; Sagot et al., 2002b) whereas profilin mutants unable to bind to FH1-like polyproline sequences have no effect (Pring et al., 2003; Sagot et al., 2002b). Furthermore, profilin-actin enhances nucleation by Bni1p FH1-FH2 but not by FH2 alone (Pring et al., 2003), which suggests that the interaction between the FH1 domain and profilin is required for increased nucleation. Thus, the FH1 domain may recruit profilin-actin and deliver the actin to the FH2 domain (Fig. 5C) just as the WH2 domain of WASP-family proteins and ActA is thought to present the actin monomer to the Arp2/3 complex (Higgs and Pollard, 2001; Yang et al., 2000). Since profilin has been shown to promote barbed-end elongation of actin filaments, profilin could also increase the polymerization rate of the filaments nucleated by the FH2 domain. Thus, profilin may have two roles in formin-dependent actin assembly: nucleation and elongation of actin filaments.

Formins may nucleate different actin bundle structures

Although the details of FH2-induced nucleation of actin

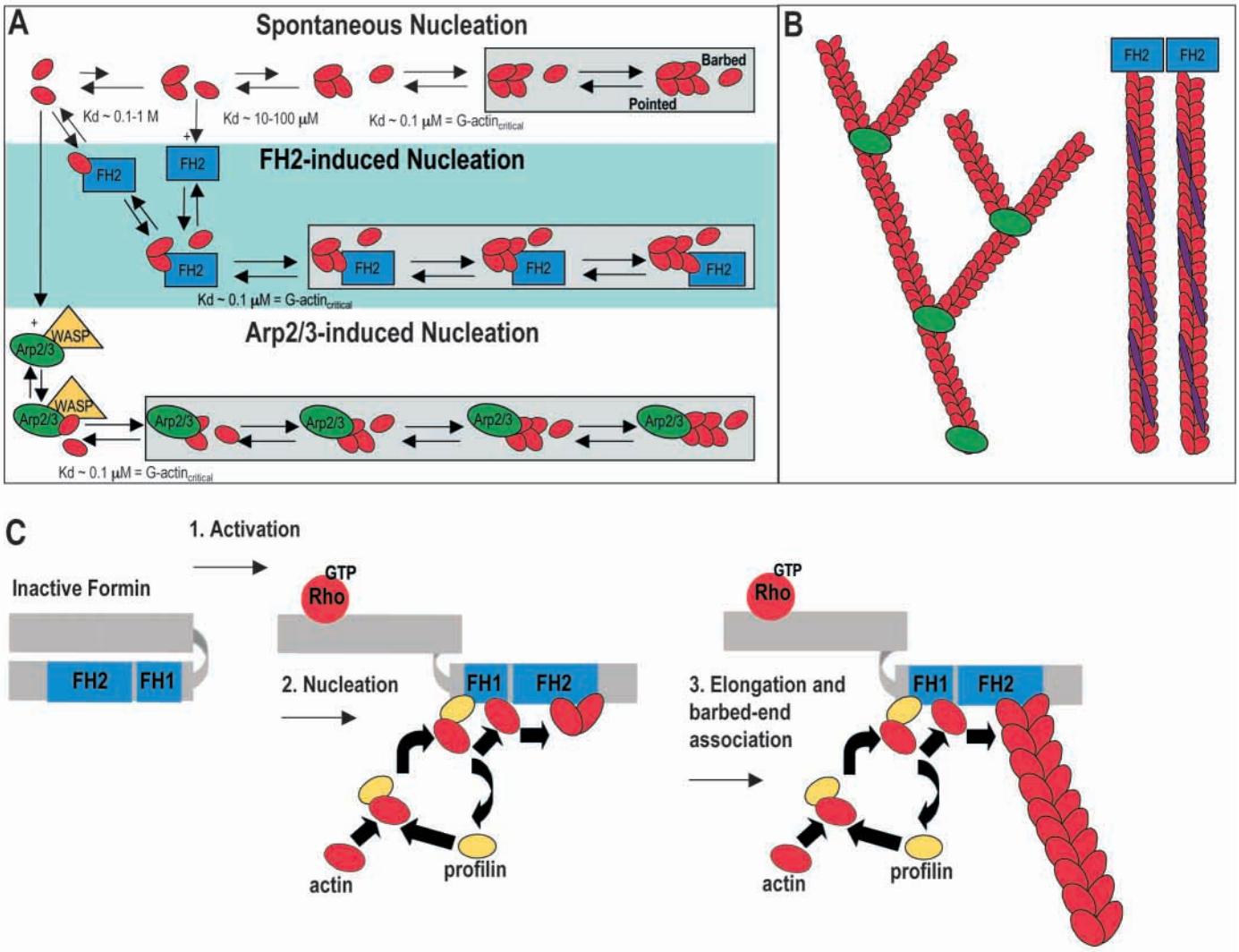

Fig. 5. (A) Two different actin nucleators. Actin nucleation by FH2 occurs by dimer stabilization. Comparison between Arp2/3-mediated nucleation and FH2-mediated nucleation indicates that the Arp2/3 complex, in its activated state, is a more efficient nucleator since it only requires the addition of one monomer to create a stable nucleus. (B) The Arp2/3 complex (left panel, green ovals) nucleates a new filament on the side of a pre-existing filament at a 70° angle. The Arp2/3 complex also caps the slowing-growing end of the new filament and allows it to grow from its barbed end, ultimately generating a branched network that is crosslinked by the Arp2/3 complex. The FH2 domain (right panel, blue rectangle) of Bni1p nucleates an actin filament but stays associated with the barbed growing end, thereby regulating filament elongation. The filaments that form are unbranched, polarized by the FH2 domain and stabilized by tropomyosin (purple). (C) Model for how the FH1 domain and profilin-actin contributes to formin-induced nucleation of actin filaments. First, the FH1 domain localizes profilin, which sequesters an actin monomer, to the nucleation vicinity. The FH1 domain may cause profilin to release its bound actin, which the FH2 domain can then utilize to nucleate an actin filament. The filament elongates at the barbed end, a process that also requires profilin-actin.

filaments remain to be elucidated, conservation of the FH2 domain suggests that formins nucleate a wide variety of actin bundles in many organisms. For instance, one mammalian formin, DFNA1, is implicated in non-syndromic deafness (Lynch et al., 1997), which suggests that it is involved in the formation of actin arrays in hair cell stereocilia. In cultured mammalian fibroblasts, the formin mDia1 is required for formation of Rho-induced stress fibers (Nakano et al., 1999; Tominaga et al., 2000; Watanabe et al., 1997; Watanabe et al., 1999). hDia, the human homolog of diaphanous, interacts with YWK-II, a structural component of human sperm membrane required for acrosomal process formation (Zhang et al., 2001).

A general requirement for formins in cytokinesis indicates that formins might control the formation of the actin contractile ring (reviewed in Frazier and Field, 1997; Wasserman, 1998; Zeller et al., 1999). *C. elegans* studies using RNAi to reduce the levels of formin CYK-1, for example, show that formins might have a direct role in this process, whereas Arp2/3 is dispensable (Severson et al., 2002). This finding has been corroborated in *Drosophila*: mutations in fly Arp2/3 or its activators are not required for formation of the cytokinetic contractile rings (Hudson and Cooley, 2002). Assembly of the budding yeast actin contractile ring involves Rho1-dependent stimulation of formin proteins and profilin (Tolliday et al., 2002). In fission yeast, the formin Cdc12 is a component of the cell division ring (Chang et al., 1997) and is required for the

formation of the leading F-actin cable that forms the actin contractile ring (Arai and Mabuchi, 2002). However, in this case, Cdc12 and the Arp2/3 complex appear to collaborate in the continual synthesis of actin filaments required for cytokinesis (Pelham and Chang, 2002). Formation of some actin structures may therefore require coordinated nucleation by both formins and Arp2/3.

Roles for formins in microtubule organization

In budding yeast, formin proteins indirectly control spindle orientation through the assembly of actin cables. Spindle orientation depends on the myosin V motor, Myo2p, which delivers the protein Kar9p to the tip of the growing bud (Beach et al., 1999; Yin et al., 2000). Kar9p functions as a cortical anchor for Bim1p, which binds to the ends of the cytoplasmic microtubules (Korinek et al., 2000; Lee et al., 2000; Miller et al., 2000). Thus, the Myo2p-Kar9p-Bim1p complex provides a physical link between the mitotic spindle and an actin cable and, since Bni1p nucleates and polarizes actin cables, it controls mitotic spindle orientation indirectly (Fig. 4). In the developing mouse oocyte, the role of formin 2 resembles that of yeast Bni1p: it is required for actin-dependent spindle positioning and the establishment of cell polarity (Leader et al., 2002).

In mammalian cells, mDia formins modulate microtubule function and organization (Isizaki et al., 2001; Kato et al., 2001; Palazzo et al., 2001). Overexpression of an activated form of mDia1 induces bipolar elongation of HeLa cells and alignment of microtubules and F-actin bundles with the long axis of the cell (Ishizaki et al., 2001). Interestingly, mutation of conserved residues within the FH2 domain abolishes this phenotype. Furthermore, microinjection of serum-starved NIH 3T3 cells with DNA encoding a constitutively active form of mDia2 or DNA encoding the DAD autoinhibitory domain, which leads to activation of endogenous mDia1, stimulates the formation of stable microtubules oriented towards the wound site (Palazzo et al., 2001). In addition, mDia2 colocalizes with a subset of microtubules in some cells and binds directly to microtubules in vitro (Palazzo et al., 2001). Finally, mDia1 localizes to the mitotic spindle in HeLa cells in an FH3-domain-dependent manner (Kato et al., 2001). Thus, there is a clear functional link between mDia and regulation of the microtubule cytoskeleton; however, whether the effects on microtubules depend on actin nucleation by formins remains to be clarified.

Outlook

Although roles for formin proteins in morphogenesis, cell polarity and cytokinesis have been known for some time (Wasserman, 1998; Ridley, 1999; Zeller et al., 1999), only recently has a biochemical role for formins, in the nucleation of actin filaments, been identified. This finding was a major advance because the Arp2/3 complex was the only other biologically relevant nucleator characterized previously. The regulation and molecular mechanisms that control formin-mediated nucleation remain to be elucidated in detail. The finding that formin proteins can associate with the growing barbed end of a filament is particularly interesting because this unique property would enable these morphogenetic determinants to localize the growing end of a filament to specific positions within the cell. We now need to know how formins can associate with the barbed end while still allowing monomer assembly. Structural analysis of the formin FH2 domain may provide critical information required for dissecting the residues critical for nucleation, barbed-end association and/or partial capping.

Additional actin nucleators may be identified with the use of conditional mutants in which both Arp2/3 complex and formin function are defective. The VASP/Mena family of proteins (Krause et al., 2003), for example, may function as actin nucleators in vivo; these proteins resemble formins because they can nucleate actin filaments in vitro (Huttelmaier et al., 1999; Walders-Harbeck et al., 2002), albeit inefficiently, and bind the barbed ends of actin filaments (Bear et al., 2002). Moreover, the *Listeria* protein ActA, which activates the Arp2/3 complex, has Arp2/3-independent nucleating activity that requires VASP (Fradelizi et al., 2001; Skoble et al., 2001). Human zyxin is another potential nucleator, since it appears to be able to generate new actin structures independently of the Arp2/3 complex (Fradelizi et al., 2001). A major challenge is to link each filamentous actin structure to a specific nucleation system.

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