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Formin-induced nucleation of actin filaments

Sally H Zigmond

Formins are proteins best defined by the presence of the unique, highly conserved formin homology domain 2 (FH2). FH2 is necessary and sufficient to nucleate an actin filament *in vitro*. The FH2 domain also binds to the filament's barbed end, modulating its elongation and protecting it from capping proteins. FH2 itself appears to be a processive cap that walks with the barbed end as it elongates.

Addresses
Biology Department, University of Pennsylvania, Philadelphia, PA 19104-6018, USA
e-mail: szigmond@sas.upenn.edu

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Abbreviations

DAD	Diaphanous autoregulatory domain
FH	Formin homology domain
GBD	GTPase binding domain
hDia	human homologue of *Drosophila* Diaphanous
mDia	mouse homologue of *Drosophila* Diaphanous
WASP	Wiscott–Aldrich syndrome protein

Introduction

Formins are named for the mouse gene *limb deformity* (ld), the first formin gene identified. Subsequently many genes containing the unique, highly conserved formin homology domain 2 (FH2) have been identified [1•]. Recent interest in formins has been aroused by three key findings. The first finding is that the induction of actin stress fibers by Rho requires a formin, mDia1 [2]. Second, certain actin-containing structures in yeast develop independently of Arp2/3 but require a formin [3–5]. Third, actin filaments are nucleated by incubation of G-actin with a formin's FH2 domain [6•–9•]. Apparently formins, as well as Arp2/3, can nucleate filaments *in vivo*, but the filaments serve different structures. One now wonders how formins, which contain no actin-related proteins, are able to nucleate filaments. Furthermore, what regulates formin activity to produce filaments appropriate to the time and place? Finally, why does the cell need two nucleators?

This article briefly reviews formin structure, regulation and function (for more complete reviews see [1•,10•,11,12]). I then consider new experiments on FH2-induced nucleation and FH2 binding to the filament's barbed end, modulating its elongation and protecting it from other capping proteins. FH2 itself appears to be a processive cap that walks with the barbed end as it elongates. Because the FH2 domain is highly conserved, its effects on actin probably contribute *in vivo* to many formin-dependent structures including the cleavage furrow, actin cables and stress fibers. Here I propose that the filaments nucleated by formins serve different mechanical functions from those nucleated by the Arp2/3 complex: formin-nucleated filaments may sustain tension for contraction, whereas Arp2/3-nucleated filaments may sustain compression for protrusion.

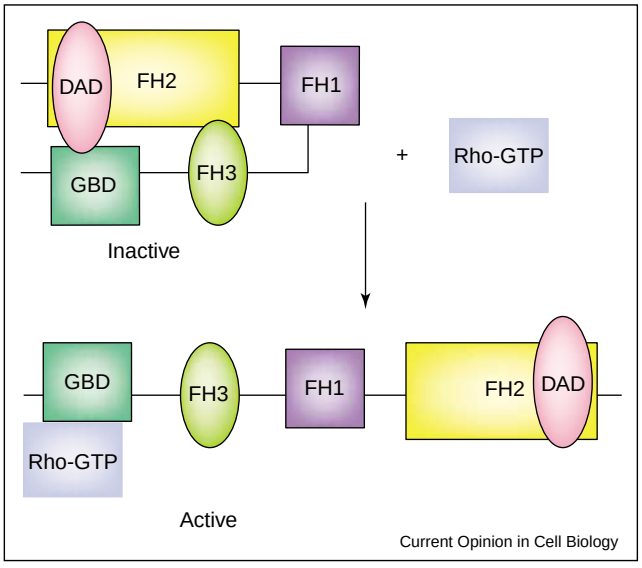
Structure, localization and regulation of formins

Formins are multi-domain proteins defined by strongly conserved FH2 domains (Figure 1) [1•]. The FH2 domain of Bni1, when crystallized, forms a dimer, and a slightly longer version in solution forms a tetramer (M Eck, personal communication [9•,13•]. When incubated *in vitro* with pure actin, FH2 is necessary and sufficient to nucleate actin [6•–9•]. The FH2 domain is usually flanked on the N-terminal side by an FH1 domain. The latter is proline-rich and binds profilin, SH3 domains (including those of the Src family, IRSp53, and DIP) and WW domains [12,14•,15–17]. The FH1–FH2 region when expressed in a cell functions as a constitutively active formin.

Formin function is regulated by factors that bind to additional domains. For example, in formins related to Diaphanous, a Rho GTPase binds to an N-terminal GTPase binding domain (GBD). This binding reduces the inhibitory interaction between the GBD and a C-terminal DAD (Diaphanous autoregulatory domain) [1•,9•,10•,18]. Deletion of either the GBD or the DAD creates a consitutively active formin [2,14•,18]. The particular Rho GTPase that activates Diaphanous-related formins varies [2,19•–21•,22,23]. Some formins lack a recognizable GBD and their regulation remains obscure [1•,10•].

Formin localization in the cell depends on additional domains. N-terminal domains such as FH3 [10•] and PDZ anchor some formins to a subcellular structure; for example, a PDZ domain anchors delphilin to the post-synaptic membrane of Purkinje cells [24]. Plant formins can be sorted into two subfamilies defined by the presence or absence of an N-terminal transmembrane domain [25]. In some cases, formin activation results in formin association with the induced filaments [22].

Figure 1



Activation of Diaphanous-related formin by Rho-GTPase. Diagram of domains present in Diaphanous-related proteins, illustrating the conformation change that occurs upon binding Rho-GTP.

Formin-dependent functions vary between organisms and cell types. In most cells they contribute to polarity and cytokinesis [26–29]. In yeast, the contractile ring and actin cables form independently of Arp2/3 but require formins. These cables anchor to a particular site (e.g. the tip or neck of a bud) and serve as a highway for myosin-mediated transport of vesicles and other organelles to that site.

The mammalian genome contains at least nine formin genes [9•], and several are expressed in multiple splice variants [14•,30]. Stress fibers induced by Rho require a Rho-kinase but also a formin — mDia1 or mDia2 [2]. Fibers induced by experimentally applied tension require mDia1 but not Rho-kinase [31]. Various formins are implicated in cell movement [32,33], filopodial formation [21•], endocytosis, endosome movement [14•], meiotic spindle movement [34] and focal contacts [31], and in signaling pathways activated by Rho [35], Src [15,36], Wnt [16], insulin [30] and serum [37].

Several factors complicate the assignment of formin function *in vivo*. First, many cells express multiple formins with overlapping functions. For example, in *Saccharomyces cerevisiae*, the formin Bni1p normally produces actin cables, but in its absence another formin, Brn1p, can suffice [20•]. In mammalian cells, stress fibers can be induced either by Rho's activation of mDia1 or mDia2 or by Rac's activation of a third formin, FHOD1 [2,22,33]. Second, different members of the Rho family can affect a given formin. Thus, although Rho3 is the preferred activator of Bni1p, Rho4 can activate it sufficiently to support viability, as can overexpressed Cdc42 [20•]. Finally, formin loss or gain causes many secondary effects [21•]: formin-induced actin polymerization affects gene expression [37]; formin-induced filaments mediate stress fiber contraction that in turn can affect focal contacts and microtubule distribution [31]; and some formins can alter the level of active Rho, which in turn can affect Rac and the multiple downstream targets of these Rho GTPases [38].

FH2 mediates actin nucleation

FH2 is necessary and sufficient for nucleation [6•,9•]. The mechanism apparently involves dimer stabilization (Figure 2) [8•,9•,39•]. This contrasts with spontaneous nucleation, where the first intermediate that can elongate like a barbed end is a trimer. As FH2 domains themselves oligomerize [9•,13•], two FH2 molecules probably stabilize the actin dimer. The FH2–actin complex of dimers might arise by an FH2 dimer capturing a pre-formed actin dimer or by its sequentially binding two actin monomers. Because the concentration of free G-actin in cytoplasm is

Figure 2

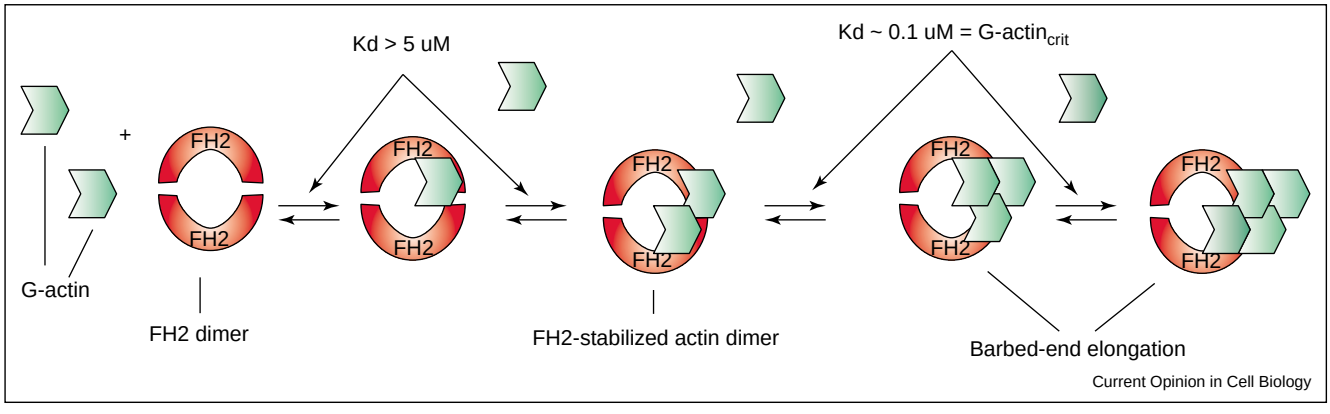


Diagram of the FH2-induced nucleation mechanism. In this diagram FH2 is illustrated as a dimer that sequentially binds to G-actins to form the stabilized dimer. The stabilized dimer has an affinity for G-actin similar to that of a filament barbed end (K_d ~0.1 uM).

low (~0.5 μM) and actin dimers are very unstable, the concentration of spontaneously formed dimers is extremely low [40]. Thus, the most likely mechanism is sequential binding of monomers [39•].

The affinity of FH2 for G-actin and the affinity of the formin–actin complex for an additional actin are both low (<5 μM), so FH2-induced nucleation in 0.5 μM G-actin is very slow. However, *in vivo*, formin-induced filaments turn over rapidly [27], suggesting that additional factors accelerate nucleation. Profilin is an essential cofactor *in vivo* for many formin-dependent functions [26,28]. Profilin binds *in vitro* to FH1, allowing profilin–actin to be a substrate for nucleation, although it is less effective at enabling nucleation than free G-actin [9•,39•,41]. *In vivo*, profilin–actin is present at a much higher concentration than free G-actin [42], and is therefore probably the primary substrate for nucleation. Furthermore, the high ratio of profilin–actin to free G-actin implies that profilin–actin accounts for most elongation. For filaments induced by Cdc12, profilin–actin's contribution is even more profound, because Cdc12 severely inhibits elongation by free G-actin but not by profilin–actin ([8•]; see also below).

Other factors may increase formin's affinity for G-actin and/or deliver G-actin to the formin just as VCA (the C-terminus of the Wiscott–Aldrich syndrome protein, WASP) delivers G-actin to Arp2/3. Contributors to nucleation may include the following: Bud6p/Aip3p, a yeast actin-interacting protein [43]; VASP, which is required for mDia-induced increase in F-actin [44]; DIP (Diaphanous interacting protein) and components of the Src and Wnt signaling pathways, which bind to FH1 [36]; Rac, which binds to part of FH1 in the formin FHOD1; and elongation factor 1α, which binds to a domain between FH1 and FH2 [1•,45].

FH2 caps the barbed end and protects it from capping by capZ homologues

In vitro, the FH2 domain of Bni1 binds selectively to the barbed end. This is not explained by the fact that actin subunits at the polymerizing barbed end contain ATP or ADP-Pi, as the formin also partially inhibits depolymerization when the actin subunits contain ADP [9•,39•]. Apparently FH2 prefers a binding surface only available at the barbed end.

FH2 at the barbed end inhibits the rates of both polymerization and depolymerization. This inhibition is only partial even when the FH2 concentration is high enough to saturate all the barbed ends [6•,9•,39•]. Thus, FH2 is not a weak capper (one that binds with low affinity but when bound totally inhibits barbed-end dynamics); rather, it is a 'leaky' capper, allowing polymerization even while bound to the end [6•,9•,39•]. Different formins inhibit elongation to different degrees. For example, barbed-end elongation with G-actin is inhibited ~50%

Figure 3

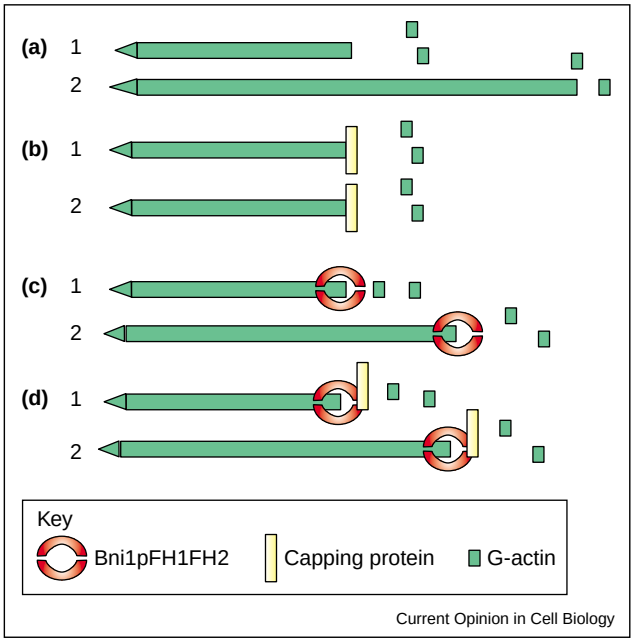


Diagram showing properties of the Bni1pFH1FH2 barbed-end leaky cap. Bni1pFH1FH2 slows elongation but protects the barbed end from capping. Each panel 2 represents a later time point relative to panel 1. **(a)** Normal elongation with G-actin in the absence of capping protein or Bni1pFH1FH2. **(b)** Capping protein is a 'tight capper' that allows no elongation when bound. **(c)** Bni1pFH1FH2 is shown as a dimer that partially inhibits elongation by G-actin (and by profilin–actin, not shown), acting as a 'leaky cap'. **(d)** Bni1pFH1FH2 at the barbed end protects that end from capping protein, allowing elongation even in the presence of capping protein.

by Bni1p–FH2 [6•] and ~90% by Cdc12p–FH2. However, if the FH1 domain is present, elongation with profilin–actin is still inhibited ~50% by Bni1p but not at all by Cdc12 [8•] (Figures 3 and 4). Even more surprisingly, profilin relieves Cdc12p's inhibition of barbed-end depolymerization (Figure 4). How profilin relieves the inhibition remains unclear, but the mechanism is not complete removal of the Cdc12 from the barbed end, as filament annealing remains blocked [8•].

FH2 at the barbed end competes with high-affinity capping proteins such as capZ homologues and gelsolin [13•]. At steady state, the fraction of ends bound by each capper will depend on the capper's relative concentration and affinity; thus, in the bulk cytoplasm, cappers with very high affinities may prevail. However, formins may dominate at sites where they are highly concentrated; for example, Bni1p is highly concentrated at the bud tip of yeast cells. In any case, since a filament nucleated by a formin retains that formin at its barbed end for some time, its elongation must continue for longer than a free barbed end. This contributes to formin's ability to increase the F-actin level.

Figure 4

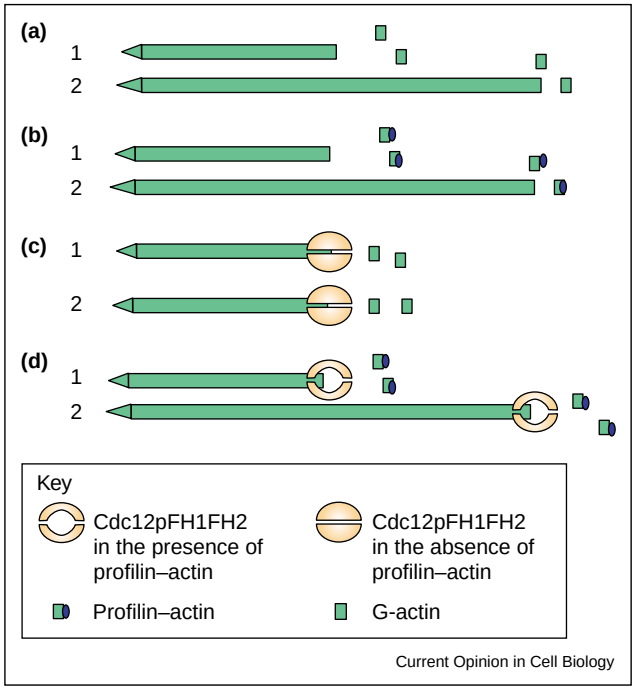


Diagram showing properties of Cdc12pFH1FH2. **(a)** Normal elongation with G-actin in the absence of Cdc12pFH1FH2. **(b)** Normal elongation with profilin–actin in the absence of Cdc12pFH1FH2. **(c)** Cdc12pFH1FH2 severely inhibits elongation with G-actin but **(d)** allows normal elongation with profilin–actin. Although the mechanism is unknown, in this cartoon the binding of profilin to FH1 increase the opening between the two formins, allowing the actin monomer access to the barbed end.

How does a leaky cap work?

The leaky cap produced by FH2 (Bni1p) can be maintained even while the filament elongates at >30 monomers per second. This cannot be explained merely by FH2's rapid 'on' and 'off' rate at the barbed end [13•,46]; rather, FH2 appears to be a processive cap that walks with the end as it elongates (Figures 3 and 4; see also animation in [13•]). This action probably depends on FH2's ability to dimerize or multimerize [9•,13•]. As the barbed end binds the next monomer, the actin that binds FH2 goes from an 'end' to an 'internal' position, which decreases its affinity for FH2. But as this FH2 releases, the second FH2 remains attached to the filament, allowing the free FH2 to reattach to the new end. Because the 'walk' is effectively an internal rearrangement of an existing molecular complex, its 'on' rate is independent of bulk fluid diffusion; thus, it should have first-order kinetics with no theoretical limit.

Actin nucleation can explain most formin functions *in vivo*

The properties of the FH1–FH2 fragment *in vitro* appear to account for formin functions *in vivo*. Best understood is the budding yeast formin Bni1p, which appears to simultaneously nucleate a filament and anchor its barbed end to the bud tip. This anchored filament can still elongate at ~100 monomers per second by inserting actin monomers at the bud tip [13•,47]. The actin cables so produced, bound by tropomyosin and cross-linked by fimbrin, function in the polarized transport of vesicles toward the bud, nuclear positioning and spindle orientation [4,5,10•,27–29,48,49,50•,51]. Similarly, in mammalian cells formin-induced nucleation evoked by Rho explains the *in vivo* requirement of formins for stress fiber formation [26,52]. Formin-induced nucleation appears necessary for contractile ring formation in most species. Furthermore, there is evidence in *Drosophila* and yeast that regulators of Rho determine both the timing and location of cytokinesis [28,53,54]. Finally, in mammalian cells, other formin-dependent functions including cell elongation, alignment of microtubules and maturation of focal contacts may all depend on the induction of stress fibers by Diaphanous [31,55].

Other formin-dependent functions, although less well understood, mostly involve F-actin: these functions include the formation of adherens junctions between epithelial cells [56], microtubule-independent movement of meiotic chromosomes [34], cell migration [33], movement of early endosomes along actin filaments [14•], and formation of filopodia, microspikes and lamellipodia [21•,33]. Even activation of the serum response factor [15,36,37] requires formin-induced actin polymerization. In addition, formins serve as adaptors to assemble various signaling proteins [10•]. Some functions ascribed to particular formins are likely to be incorrect [10•]. These include the following: DFNA1 in stereocilia formation (since the deafness is not congenital but rather arises from endolymphatic hydrops in childhood [57]); hDia in sperm acrosome formation (the sperm component interacting with hDia is extracellular [58]); and Formin1 in limb deformity (the defect is probably due to a mutation in gremlin [59]). Of course, formins may also have downstream effectors other than actin.

In certain cases, formins localize specifically to their site of action. Thus, in budding yeast, a formin concentrates at the bud tip (Bni1p) and at the bud neck (Bnr1p), and both Bni1p and Bnr1p concentrate at the contractile ring [10•,26,52]. In mammalian cells, formins concentrate with cleavage furrows, where their function is obvious. On the other hand, they are also found at surface membranes, endosomes, phagocytic cups, microtubule organizing centers, stable microtubules, mitotic spindles and midbodies, where their function is not obvious [14•,21•,60,61]. Nor is it always clear if concentrated formin is activated. While evoking stress fibers, mDia1 does not noticeably localize with them [2,31], possibly because active mDia1 is a small fraction of the total. FHOD1, which also evokes stress fibers, does localize with them but only when activated [22,33].

Why two nucleators?

The Arp2/3 complex and formins both nucleate actin filaments, but their different mechanisms generate different patterns. Arp2/3 is activated by an existing filament to nucleate a branch from the parent filament, with Arp2/3 at the branch point. This creates a dendritic network of filaments. Formins nucleate from monomers alone and this generates straight filaments. Formin-nucleated filaments *in vivo* are often bundled, but because branches created by Arp2/3 do not persist, Arp2/3-nucleated filaments also become bundled. Thus, bundled filaments can arise from either mechanism.

Actin filaments nucleated by Arp2/3 and formins may also serve different mechanisms in cell movement. First, there is cell protrusion, which is driven by actin polymerization. A filament pushes on the cell surface as it elongates. This mechanism, which also propels certain intracellular vesicles and pathogens, is mediated by Arp2/3, whose dendritic network is apparently optimized for elongation to exert a protrusive force [62].

The second mechanism is contraction, which is driven by the movement of myosin along the actin filament. Contraction (and also transport powered by myosin) uses formin-induced actin structures such as stress fibers, contractile rings and yeast cables [50•]. Filaments in these structures are coated by tropomyosin, which enhances their tensile strength [63]. Furthermore, their barbed ends are bound from the time of nucleation by a formin which, in turn, can be anchored by accessory proteins to a particular site (e.g. to the bud). Thus strengthened and anchored, the new filament can bear tension during contraction. Consistent with this general division of labor, Rac and Cdc42 contribute to the protrusion of lamellipodia and filopodia by activating (via the WASP family) Arp2/3, whereas Rho serves contraction and transport by activating formins and myosin. By independently activating formins to nucleate actin, and myosin to mediate contraction, Rho coordinates both assembly and function of contractile structures [35].

Protrusive structures such as lamellipodia contain formins as well as the expected Arp2/3 [31,64]. This may suggest that formins contribute to protrusion. Possibly, but the protruded structures are also contractile; thus, myosin mediates rearward movement of the lamellar F-actin, and exploratory filopodia, subsequent to attachment, pull the cell forward [21•,65–67]. Thus, here too filaments nucleated by Arp2/3 and formins may serve different mechanical functions.

Once polymerized, the actin filaments produced by either pathway are identical. Therefore, their functions may shift and merge. Filaments nucleated by Arp2/3 are eventually coated with tropomyosin, crosslinked and rearranged by contractile forces to form actin arcs. Conversely, actin filaments nucleated by formins can be bundled and supported by cross-linkers to mediate protrusion, possibly contributing to the movement of actin cables or filopodial extension [21•,47]. Whether the proposed dichotomy holds up, and whether additional nucleators also contribute, further study will reveal. It will be particularly interesting to learn how actin filaments are nucleated in structures where they provide skeletal support rather than movement, for example in intestinal microvilli, auditory hair cells and *Drosophila* bristles.

Conclusions and future directions

Currently formins fit a simple conceptual model: they are activated, often downstream of a Rho GTPase, to nucleate actin filaments anchored at the barbed end. Formin-induced filaments appear to serve a different mechanical function from Arp2/3-nucleated filaments. Whereas the Arp2/3-induced dendritic network is optimized for protrusion, formin-induced filaments function with myosin (independently activated by Rho) to support contraction and organelle transport. Many cell features, including polarity, focal contacts, microtubule distribution and vesicle trafficking, may depend on this basic formin function.

How general is this model? The formin family, being large and complicated, is only partially understood. Our best understanding of *in vivo* function rests on genetic experiments in yeast, where both Arp2/3 components and formins can be deleted and replaced by recombinant constructs. Initial genetic experiments in *C. elegans* and *Drosophila* are also informative but in mammalian cells the tools for dissecting formin and Arp2/3 function are limited. Most mammalian studies demonstrate that an active formin can replace a particular agonist (e.g. Rho, serum) in a signaling pathway. However, the RNAi and knockout experiments that are starting to be carried out will provide new insights [21•]. FRET studies will also help monitor the activation of formins and Arp2/3 as well as their interactions with other proteins [21•]. Biochemical studies are needed to characterize *in vitro* the properties of the whole protein, including its structure and its interactions with other proteins.

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- •• of outstanding interest

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