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Curr Opin Cell Biol. Author manuscript; available in PMC 2010 February 1.

Published in final edited form as:
Curr Opin Cell Biol. 2009 February ; 21(1): 28–37. doi:10.1016/j.ceb.2008.12.001.

Actin Nucleation and Elongation Factors: Mechanisms and Interplay

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Abstract

Cells require actin nucleators to catalyze the *de novo* assembly of filaments and actin elongation factors to control the rate and extent of polymerization. Nucleation and elongation factors identified to date include Arp2/3 complex, formins, Ena/VASP, and newcomers Spire, Cobl, and Lmod. Here, we discuss recent advances in understanding their activities and mechanisms, and new evidence for their cooperation and interaction *in vivo*. Earlier models had suggested that different nucleators function independently to assemble distinct actin arrays. However, more recent observations indicate that the construction of most cellular actin networks depends on the activities of multiple actin-assembly promoting factors working in concert.

Introduction

Many cellular processes powered by actin polymerization (e.g. cell motility, endocytosis, and cytokinesis) depend on responsive, rapid bursts of actin filament assembly at specific subcellular locations. Cells typically contain a large pool of actin monomers that is buffered by actin monomer-binding proteins such as thymosin β4 and profilin. These factors suppress spontaneous nucleation of new filaments, yet enable rapid mobilization of monomers for elongation at existing filament ends. This makes nucleation the rate-limiting step in *de novo* filament formation. Once nucleated, filaments elongate at their fast-growing (barbed) ends at a rate linearly proportional to the concentration of available actin monomers. Elongation at the slower-growing (pointed) ends of filaments may not be physiologically relevant since most actin monomers are bound to proteins that block addition to pointed ends. The extent of filament elongation *in vivo* is severely limited by the presence of high affinity barbed end capping proteins.

To overcome these barriers to filament nucleation and elongation, cells express actin assembly-promoting factors. First, a variety of actin nucleators with distinct mechanisms respond to cellular signals and regulate the precise timing and location of filament formation. Second, actin elongation factors control the extent of filament growth by protecting barbed ends from capping proteins and influence the rate of actin subunit addition. By employing specific combinations of nucleators and elongation factors, each with distinct mechanisms and modes of regulation, cells gain the versatility required to construct actin networks with specialized architectures and functions.

In this review, we compare the biochemical mechanisms of different actin nucleators and elongation factors, then consider how these activities are used in different combinations to

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generate cellular actin structures *in vivo*. Finally, we examine how emerging scaffolding proteins coordinate the spatial and temporal activities of multiple actin assembly factors.

Actin Nucleators

What are the properties of a *bona fide* actin nucleator? A nucleator can be defined as a factor that stimulates formation of a filament that grows rapidly at its barbed end. In addition, a nucleator should be able to efficiently seed polymerization from a pool of profilin-bound actin monomers (profilin-actin), since this may be the dominant species of available ATP-actin monomers in eukaryotic cells. Spontaneous filament assembly involves sequential formation of highly unstable polymerization intermediates (actin dimers and trimers) that rapidly dissociate, making spontaneous nucleation highly inefficient. In principle, an actin nucleator could use one of three mechanisms to surmount this barrier: (1) structural mimicry of polymerization intermediates, (2) stabilization of spontaneously formed intermediates, or (3) recruitment and alignment of actin monomers to form a polymerization 'seed'. Nucleators have now been identified that utilize each of these three mechanisms (Figure 1a).

The first nucleator identified, Arp2/3 complex, employs structural mimicry [1,2]. When combined with a 'nucleation promoting factor' (NPF), Arp2/3 complex catalyzes polymerization of a new (daughter) filament from the side of an existing (mother) filament at a 70° angle to generate a branched structure. This 'dendritic' nucleation activity is used to assemble actin structures such as *Listeria* comet tails, lamellipodia, focal adhesions, and yeast endocytic patches. The most well understood Arp2/3 complex NPFs are WASp/SCAR/WAVE family proteins, which perform at least two essential roles in nucleation. First, they trigger conformational changes in Arp2/3 complex that bring its actin-related protein subunits (Arp2 and Arp3) into close register, possibly to mimic an actin dimer. Second, they recruit 1-2 actin monomers, which is a critical step in nucleation since Arp2/3 complex alone binds very weakly to monomers.

The second group of nucleators identified, formins, catalyze the formation of linear (unbranched) actin filaments *in vitro* and assemble diverse actin structures, including stress fibers, cytokinetic actin rings, and actin cables *in vivo* [3,4]. The mechanism of actin assembly by formins involves high affinity binding of their dimeric donut-shaped FH2 domains to the barbed ends of actin filaments. The FH2 domain lacks detectable affinity for actin monomers, so it has been suggested that formins catalyze polymerization by stabilizing spontaneously formed actin dimers and/or trimers [5]. Although direct experimental evidence for this model is lacking, the idea that an FH2 dimer may stabilize short-pitch associations between two actin subunits is consistent with the co-crystal structure of FH2 bound to actin, which reveals that a single FH2 'hemi-dimer' bridges two actin subunits [6]. In contrast to Arp2/3 complex, which remains associated with the pointed end of the filament it nucleates, the formin FH2 domain remains associated with the barbed end (Figure 1a). After nucleation, the FH2 moves processively with the growing barbed end, allowing rapid insertion of new actin subunits (see below).

The more recently identified nucleators Spire, Cordon bleu (Cobl), and Leiomodin (Lmod) employ yet a third nucleation mechanism that involves actin monomer recruitment to form polymerization seeds. Spire has four tandem actin monomer-binding WASp-homology 2 (WH2) domains separated by short linkers. Electron micrographs supported by hydrodynamic and spectroscopic analyses demonstrate that Spire stably associates with four actin monomers in a prenucleation complex that resembles a short, single-stranded segment of a nascent filament [7,8] (Figure 1a). It has been suggested that Spire remains associated with either the side or the pointed end of a filament after nucleation, allowing free barbed end elongation [7]. However, another group has reported that after nucleation, Spire associates with the barbed

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end and blocks elongation by profilin-actin [8]. Thus, the post-nucleation effects of Spire have yet to be fully resolved. New insights might be gained by real time microscopy analysis of labeled Spire on single actin filaments. Regardless of the precise mechanism, genetic studies indicate that Spire, along with profilin and the formin Cappuccino, promotes the assembly of cytoplasmic actin meshworks that control cytoplasmic streaming in *Drosophila* oocytes (see below), and may have roles in membrane trafficking [9].

Cobl's nucleation mechanism is somewhat related to that of Spire, but with key differences. Nucleation by Cobl requires three actin-binding WH2 domains separated by linkers. Deletion of either the first or third WH2 domain greatly diminishes nucleation activity, whereas in Spire, deletion of individual WH2 domains only incrementally reduces activity. Moreover, one of the linkers in Cobl is substantially longer than those found in Spire [10]. Shortening this linker abolishes nucleation, and substituting an unrelated sequence of similar length restores nucleation. Thus, linker length rather than sequence appears to be critical for function. This has led to the suggestion that Cobl stabilizes both short-pitch and long-pitch associations to generate polymerization nuclei (Figure 1a), a model that should be tested through structural analyses. Cobl is highly expressed in the brain, and knock down experiments in primary hippocampal neurons demonstrate that it is required for normal levels of neurite extension and branching. It will be interesting to learn what specific actin structures are assembled by Cobl to promote neurite extension.

The third member of this nucleation group, Lmod, is expressed in cardiac muscle tissue. Nucleation by Lmod depends on a single WH2 domain and two unrelated actin-binding domains similar to those found in tropomodulin (Tmod), a pointed end filament capping protein [11]. Lmod uses this non-uniform array of actin monomer-binding domains to organize 2-3 actin monomers into a polymerization seed, possibly stabilized at its pointed end in a Tmod-like fashion. One of the interesting properties of Lmod is that its nucleation activity is stimulated *in vitro* by tropomyosin, similar to formins [12], but little else is known about how the activities of Lmod (or Cobl) are regulated.

Actin Elongation Factors

Once nucleated, filaments grow freely at their barbed ends until monomer pools are depleted and/or capping proteins terminate elongation. Because of the high association rate constant for capping proteins, which are abundant in nearly all cell types, filament lengths are severely limited *in vivo* unless their growing barbed ends are protected or capping protein is locally inactivated. In recent years, it has become evident that cells express proteins that associate and move with the growing barbed ends of filaments, shielding them from capping proteins and controlling the rate of elongation. We refer to the proteins as "actin elongation factors." Two have been characterized to date, formins and Ena/VASP.

Formins use their dimeric, donut-shaped FH2 domains to crown the barbed end and processively move with the growing filament end [4,13]. This dynamic attachment requires both halves of the formin dimer to be functional, and may involve alternating contacts of the FH2 with the two actin subunits exposed at the barbed end. However, the precise mechanism underlying processive movement remains to be determined. The adjacent FH1 domain appears to be long, unstructured, and possibly rope-like. It contains multiple polyproline tracts that recruit profilin-actin monomers, and by a poorly understood mechanism 'delivers' these complexes to the FH2-capped barbed end for rapid addition. This set of interactions can lead to FH1-FH2-dependent acceleration of barbed end elongation, by as much as 5-fold over the rate of elongation at free barbed ends [14] (Figure 1b). Further, different formins accelerate elongation to highly variable extents (1.25 – 5.0 fold). Interestingly, the requirements for formin-mediated actin nucleation and elongation are similar, both requiring two functional

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halves of an FH2 dimer with intact actin-binding sites. Thus, the same properties of the FH2 dimer that allow it to processively move with an elongating barbed end may be critical for "capture" of polymerization intermediates to nucleate actin assembly.

Enabled/vasodilator-stimulated phosphoprotein (Ena/VASP) is the other known actin elongation factor. Proteins in this family are ubiquitously expressed in mammals, localize to actin-rich zones (e.g. focal adhesions, cell-cell contacts, filopodial tips, and lamellipodia), and contribute to cell motility, axon guidance, cell adhesion, endocytosis, and intracellular pathogen motility [15,16]. Like formins, Ena/VASP proteins bind profilin-actin and multimerize, but form tetramers rather than dimers [17-19]. Unlike formins, they do not appear to nucleate actin assembly under physiological salt conditions in solution [20]. However, this issue may warrant further investigation given the recently reported distinction between Ena/VASP activities in solution versus clustered/immobilized on beads (see below). Similar to formins, Ena/VASP proteins immobilized on beads can protect barbed end growth in the presence of capping proteins [21]; however, this effect requires substantially higher concentrations of Ena/VASP compared to formins, perhaps reflecting differences in barbed end binding affinities and/or rates of dissociation. Live cell imaging experiments show that GFP-VASP remains at the ends of actively growing filopodial tips, which is consistent with either persistent or transient engagement of nascent barbed ends [22].

Recent efforts to reconcile conflicting data between the behavior of Ena/VASP in solution and on beads have suggested unique requirements for its attachment and protection activity. While bead assays had shown that Ena/VASP can protect barbed end growth in the presence of capping proteins [21], total internal reflection fluorescence (TIRF) microscopy studies monitoring individual actin filaments had suggested that elongation ceases when barbed ends become attached to mouse VASP immobilized on a glass surface [23]. The seemingly conflicting observations left it unclear whether the differences in activity were due to differences in the VASP proteins, purification procedures, and/or assay conditions. These issues appear to have been resolved by a new study, which uses TIRF to directly compare human and *Dictyostelium* VASP proteins in solution and immobilized on beads for their effects on rate of barbed end elongation, both in the presence and absence of capping protein [24]. Protected elongation was observed with VASP on beads but not in solution, suggesting that the activity requires tethering/clustering of VASP. Thus, Ena/VASP proteins may processively cap and protect filaments *in vivo* only after their spatial recruitment to cortical foci, e.g. incipient filopodial tips. This study also answers a longstanding question - do Ena/VASP proteins accelerate barbed end elongation? One previous study on mouse VASP showed that it increased rate of filament elongation by only 1.2 fold in solution [23]; however, the new TIRF study shows that human and *Dictyostelium* VASP accelerate elongation more substantially, by about 2 and 7 fold, respectively [24]. Thus, both VASP and formins can accelerate elongation, although formins require profilin for this activity. An important principle emerges from these collective studies: different formins and Ena/VASP proteins support highly variable rates of elongation. These differences may be tailored *in vivo* to optimize the assembly of specific actin structures. For example, fast elongation rates may be optimal for filopodial extension compared to cytokinetic actin ring assembly.

What is the mechanism by which VASP protects and accelerates barbed end elongation? The protein has three parts: an N-terminal EVH1 domain that links VASP to key ligands, a central proline-rich region (PRR) that contains at least three separate binding sites for profilin-actin and actin monomers, and a C-terminal EVH2 domain that interacts with F-actin possibly to cap the barbed end. A recent study solved the structures of parts of VASP bound to profilin and actin monomers, and presents an appealing model for how a relay of interactions in the PRR might deliver monomers to the growing barbed end [19]. What is clearly needed next is

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a more complete structure of the EVH2 domain, in order to understand the basis of VASP association with the barbed end of the filament.

Collaborative assembly of lammelipodia or filopodia

Early observations suggested that Arp2/3 complex and formins may build distinct sets of actin networks *in vivo* comprised of filaments with different properties: linear versus branched, protected versus unprotected [25-28]. However, new observations are challenging this view, suggesting cross-participation by multiple actin assembly-promoting factors.

One example is in the formation of lamellipodia. These flat sheet-like membrane protrusions contain a dense network of interconnected filaments with their barbed ends generally oriented toward the membrane. The abundance of branched filaments led to over-simplified models suggesting lamellipodia are assembled entirely by Arp2/3 complex without the participation of other actin-assembly promoting factors. However, it has become clear that Ena/VASP and formins contribute to lammelipodial assembly, and recent ultrastructural analyses reveal that lammelipodial actin networks include shorter and longer filaments, and filaments with heterogenous branch angles, which cannot be explained by Arp2/3-nucleated assembly alone [29]. Targeted depletion/relocation of Ena/VASP leads to networks comprised of shorter and more branched filaments, suggesting that Ena/VASP is required for assembly of the longer filaments [21]. These effects likely stem from the ability of Ena/VASP to protect barbed end elongation at the leading edge. Formins may have a similar role in regulating lammelipodial architecture. A recent study found that knock down of mDia2 led to lammelipodial networks comprised of shorter average filament length, whereas mDia2 overexpression produced an excess of long unbranched filaments [30]. Interestingly, there were also fewer lammelipodia per cell after mDia2 knock down, demonstrating that the dendritic nucleation of Arp2/3 complex alone is not sufficient to support normal levels of the flat membrane protrusions. It remains to be determined whether Ena/VASP and formins elongate separate or overlapping sets of barbed ends, and whether Ena/VASP and formins nucleate filaments or instead hijack barbed ends of Arp2/3-nucleated filaments.

A second example of cross-participation is in the formation of filopodia. These finger-like membrane protrusions contain a compact, linear bundle of long, unbranched filaments with their barbed ends oriented toward the filopodial tips. Formins and Ena/VASP localize to filopodial tips, and their genetic requirement for filopodial assembly is undisputed [30-32], but it remains uncertain why filopodial assembly requires the activities of two different actin elongation factors. One possibility is that formins play a more critical role initiating filament assembly, whereas Ena/VASP is more crucial for organizing and controlling the rate of growth of barbed ends. This requires further investigation, which could include testing the combined *in vitro* effects of Ena/VASP and formins on actin.

It has also been proposed by some groups that Arp2/3 complex makes important contributions to filopodial assembly; however, conflicting data have been reported. One set of studies showed that filopodia are unaffected by knock down of Arp2/3 complex subunits in fibroblasts and melanoma cells or by null mutants of WAVE complex subunits in *Dictyostelium* [33,34]. Further, a more recent study in carcinoma cells found that knock down of WAVE2 (which is required for lammelipodial formation) increased formin-dependent filopodial assembly, suggesting that Arp2/3 complex activity suppresses filopodial formation [35]. In agreement with this view, another recent study in HeLa cells showed that WAVE2 and Arp2/3 complex directly inhibit filopodial assembly by the formin mDia2 [36]. In this study, knock down of WAVE2 or Arp2/3 complex increased mDia2-dependent filopodia formation, whereas WAVE2 overexpression decreased filopodia. This study suggests that formation of a Dia2-WAVE2-Arp2/3 complex inhibits filopodial extension until EGF-induced activation of Cdc42

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locally dissociates the complex, allowing filopodial formation. However, this complex has not been isolated, nor have the activities predicted from this model been demonstrated. Regardless, the collective genetic observations of the studies above indicate that WAVE and Arp2/3 complex have neutral or inhibitory effects on filopodial formation.

In contrast, correlative light and electron microscopy studies on migrating melanoma cells and neurons have suggested that the linear filaments in filopodia stem directly from branched filaments in the lamellipodia [30,37,38]. This has led to the 'convergent elongation' model, which suggests that filaments nucleated by Arp2/3 complex are captured at their barbed ends and organized by Ena/VASP and/or formins. The model is supported by genetic observations showing that partial knock down of different Arp2/3 complex subunits leads to a 2-3 fold reduction in number of visible filopodia on the cell surface and a reduction in frequency of filopodial initiation [38]. Further support comes from studies showing that a purified mixture of actin, Arp2/3 complex, WASP-VCA, and fascin is sufficient to assemble densely branched networks from which Arp2/3-dependent filopodial-like bundles protrude [39]. We note that additional roles for Arp2/3 complex in this process could exist. In particular, filaments nucleated by formins (and/or Ena/VASP) at filopodial tips might extend their pointed ends into the lammelipodia and be captured by Arp2/3 complex, linking them to the sides of filaments and stabilizing the filopodia. This mechanism would invert the roles of Arp2/3 complex and formins (in nucleation and capture), providing the reciprocal mechanism to convergent elongation. If both mechanisms were at work, this would lead to bidirectional growth and capture.

The reasons for the conflicting genetic results between the two sets of studies above are not yet clear. Different cell lines and/or conditions for adhesion were used in these studies [33, 38], which might affect the dependence of filopodial stability on Arp2/3 complex activity. It has also become apparent that there are different types of filopodial extensions, which may have different molecular requirements for their assembly [40]. This underscores the point that new approaches are needed to better define the coordinated series of events involved in assembling complex actin structures. For instance, new insights might be gained by combining high resolution localization of proteins in cellular actin structures with more acute genetic or pharmacological disruption of their functions.

Coordination of actin assembly by multivalent scaffolding proteins

The observations above raise a key question - how are the activities of multiple actin assembly-promoting factors spatially and temporally coordinated to orchestrate the assembly of specific actin structures? This is achieved at least in part by multi-domain scaffolding proteins that can bind and/or regulate two or more actin assembly factors. Three such factors are IQGAP1, IRSp53, and DIP/WISH (Figure 2).

IQGAP1 is a large (350 kDa) effector of Rac1 and Cdc42 required for lamellipodial assembly [41], and uses its calponin homology domain (CHD) to directly bind the EVH1 domain of N-WASP and stimulate Arp2/3-dependent actin assembly [42]. It also binds tightly to mDia1 using a distinct domain and is required for mDia1 localization to the leading edge, but interestingly has no *in vitro* effects (with or without RhoA) on mDia1 activity [43]. It is not yet clear whether IQGAP1 can bind N-WASP and mDia1 simultaneously, or whether N-WASP binding to IQGAP1 might influence mDia1 activity.

IRSp53 is a 53 kDa BAR-related membrane-binding protein that oligomerizes and induces curvature of membranes to help promote filopodial protrusion [44,45,46]. It is also an effector of Cdc42, and through its SH3 domain binds to Ena/VASP, mDia2, WAVE2 and N-WASP. Thus, IRSp53 may concatamerize on membranes to spatially organize these actin regulators into filopodial tip complexes and coordinate membrane deformation with actin assembly.

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Consistent with this model, IRSp53 induces filopodia in an N-WASP- and Ena/VASP-dependent manner [46].

DIP/WISH is a 90 kDa protein that has an SH3 domain and binds to both N-WASP and Arp2/3 complex to stimulate Arp2/3-dependent actin assembly [47,48]. It also has a Leucine-rich repeat (LRR) domain, through which it potently inhibits the formin FH2 domain to suppress mDia2-dependent filopodial assembly [49]. Consistent with these activities, knock down of DIP/WISH impairs lamellipodial assembly [48], and over expression of DIP/WISH impairs filopodial formation [49]. Moreover, DIP/WISH localizes to both filopodial tips and the lammelipodial cortex. It is interesting that both IRSp53 and DIP/WISH localize to filopodial tips, and appear to have antagonistic roles in filopodial assembly. It remains uncertain whether these two proteins perform their functions in the same or competing complexes.

Direct interactions between formins and Spire

Actin nucleator cross-regulation can also be direct, as demonstrated by the interactions between Spire and the formin Cappuccino. This topic has been extensively reviewed elsewhere [50, 51], so it is only commented on briefly here. Genetically, Cappuccino, Spire and profilin are each required for the formation of a cytoplasmic actin network in oocytes that affects events during development [52]. The precise role of the actin meshwork is not yet fully understood, but it is required for proper regulation of cytoplasmic streaming, microtubule orientation, and cell polarity. Interestingly, loss of the actin network in *cappuccino* mutants cannot be rescued by overexpression of Spire, but loss of the network in *spire* mutants can be partially rescued by expression of an activated Cappuccino. Thus, formins may have the central role in the assembly of these networks, enhanced by Spire. In either case, the genetic data available make a strong case that Spire and Cappuccino synergize *in vivo* to assemble these actin networks, and a possible mechanism for the synergy arose from the observation that they directly interact [53].

Defining the biochemical effects of Spire-Cappuccino interactions on actin assembly has proven to be more difficult. One group reported that Spire KIND domain inhibits Cappuccino-mediated actin assembly, while this interaction activates Spire [54] (Figure 2). A different study concluded that the KIND domain has no effect on Cappuccino activity [53]. Importantly, neither study explains how such effects (inhibitory or neutral) can lead to the genetic observation that Spire is required for Cappuccino-dependent actin meshwork formation. A third study offers a clear biochemical correlation with the *in vivo* observations, showing that Spire and Cappuccino synergize in a biomimetic *in vitro* motility assay [8]. However, the mechanism used to explain the observed synergy in this study involves Spire capping barbed ends, which contradicts earlier reports suggesting that Spire caps pointed ends [7]. Clearly, more work is needed to resolve the mechanism of this interaction. It may be informative to use real-time microscopy on individual actin filaments and labeled Spire and Cappuccino to determine if they act sequentially or simultaneously, and at which end of the filament.

Homeostatic cross-talk

The activities of actin assembly-promoting factors can also be balanced by homeostatic mechanisms. One recent study in *S. cerevisiae* showed that the lethality caused by an overactive formin construct could be rescued by overexpression of yeast WASp or actin monomer binding proteins [55]. The implication of these data is that formins and WASp-Arp2/3 complex compete for a limited pool of actin monomers in cells, and formin hyper-activity can be rescued by up-regulation of WASp-Arp2/3 because it restores the balance of assembly activities between these two nucleator systems. Thus, one important mechanism for maintaining proper homeostasis of actin regulation in cells is the balanced expression and activation of different nucleators. Further evidence for homeostatic mechanisms is provided by a recent study

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showing that combined knock down of N-WASP and WAVE2 leads to increased RhoA activity, which induces mDia1-dependent formation of stress fibers and filopodia [35]. These data suggest that normal expression of N-WASP and WAVE2 represses RhoA, and therefore inhibits mDia1. Consistent with these observations, another recent study reported that knock down of Arp2/3 complex subunits in B35 neuroblastoma cells led to increased RhoA activity, which in turn caused excessive formation of actin bundles and focal adhesions [38]. Although it is not yet clear how the expression of N-WASP/WAVE2-Arp2/3 machinery alters RhoA activity levels, these observations provide examples of how nucleator cross-talk can be achieved in the absence of physical interactions. Together, these studies point to another mechanism for cross-talk between actin assembly factors, complementary to the mechanisms based on physical associations described in the sections above. These findings stress the importance of considering homeostatic effects when interpreting all phenotypes arising from genetic depletion of an actin regulator.

Perspectives

A new concept coming into focus is that cells express a wide variety of actin assembly-promoting factors, and that many of them associate in functional complexes to build actin structures. There are undoubtedly more actin nucleators and elongation factors to discover, possibly with new and unique mechanisms to add to the complexity of actin regulation. A question that arises is why so many? One answer is functional diversity, to match the immense diversity in architecture of actin structures observed *in vivo*. A second answer is that the construction of even a single, highly complex actin network requires the signature activities of multiple actin assembly factors working in concert. Future investigations are likely to reveal many more cellular processes that depend on cooperation and cross-talk among actin assembly-promoting factors. The emerging complexity of these systems emphasizes that gaining a solid understanding of the cellular functions of any individual actin regulatory protein will require studying its effects in the context of the multi-protein complexes it forms *in vivo*.

Acknowledgements

We apologize to those authors whose important work we were unable to cite due to space restrictions. We thank James Moseley and Avital Rodal for critical reading of the review. Work in the authors' laboratory is supported in part by grants from the National Institutes of Health (GM063691 and GM083137).

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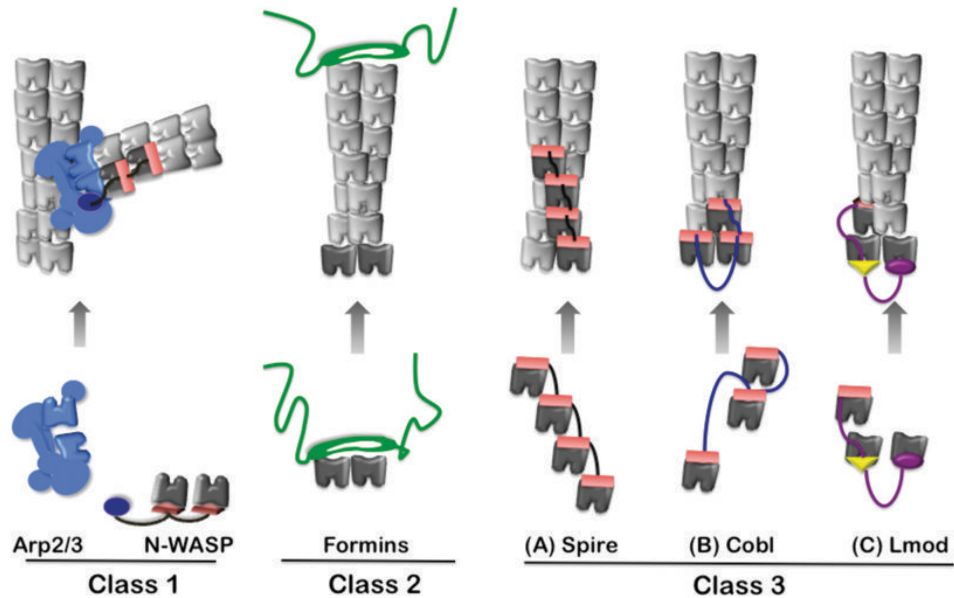

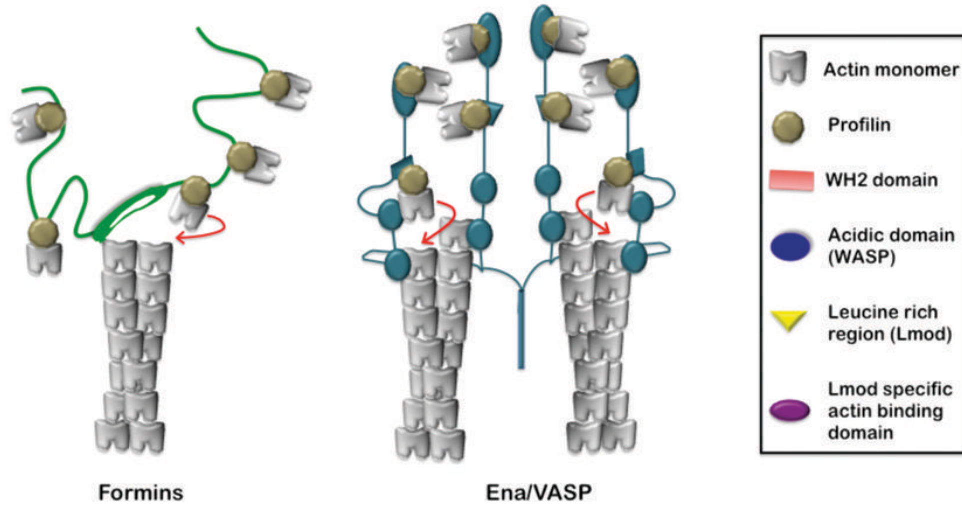

Figure 1. Proposed mechanisms of actin assembly factors

(a) Three classes of actin nucleators. Nucleator domains are displayed in color, actin subunits used by nucleators to seed polymerization in black, and actin subunits polymerized from nuclei in grey. Class I: N-WASP uses its WH2 domain(s) to recruit actin monomers and its acidic (A) domain to bind to an actin-related protein subunit of Arp2/3 complex. This structure stabilized by N-WASp may mimic an actin trimer. Class II: formins are hypothesized to nucleate actin polymerization by stabilizing spontaneously formed actin dimers and/or trimers. Formins remain associated with the barbed end while permitting addition of actin subunits. Class III: Spire, Cobl and Lmod contain between one and four WH2 domains each, separated by intervening linker sequences of variable length. Their nucleation mechanisms are related,

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but each may generate an actin nucleus with distinct properties, stabilized by lateral and/or longitudinal contacts between subunits, and in some cases capped at one end. Note, in some respects N-WASp represents a specialized form of Class III nucleator, in which the third actin monomer-binding domain has been replaced with a domain that binds to actin-related proteins. **(b)** Actin elongation factors. Formins shield barbed end growth from capping proteins by using their dimeric FH2 domains to processively move with the filament end. Adjacent rope-like FH1 domains are used as “arms” to recruit profilin-actin complexes and ‘deliver’ them to the FH2-capped filament end for rapid addition. The elongation mechanism of Ena/VASP is not well understood. However, it tetramerizes, bundles filaments, and may engage multiple barbed ends simultaneously. Its ability to accelerate barbed end elongation could involve a relay or hand-off of actin monomers using multiple actin-binding domains (adapted from model in [19]).

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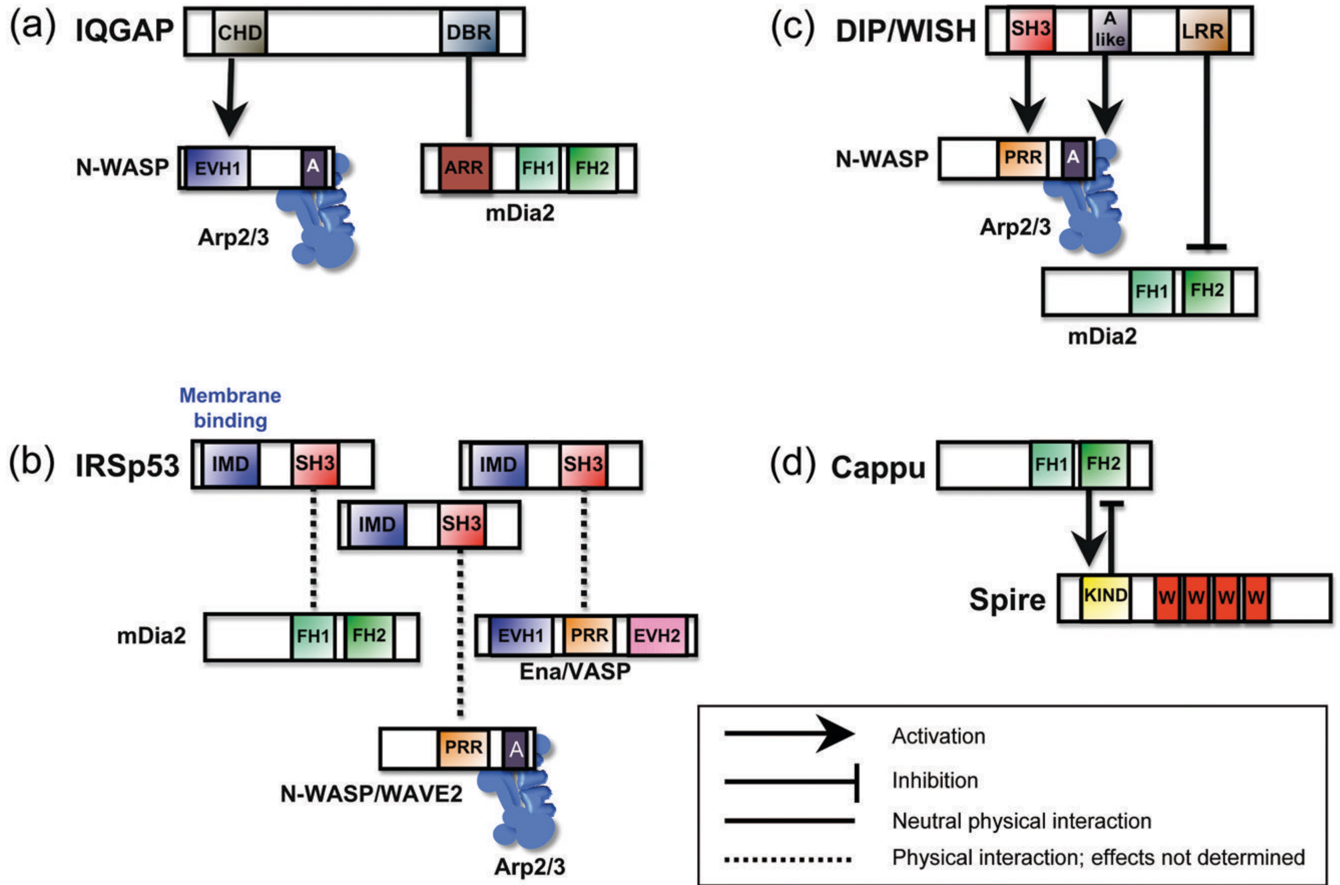

Figure 2. Physical complexes that spatially and temporally coordinate multiple actin assembly factors

(a) IQGAP uses its calponin homology domain (CHD) to associate with the EVH1 domain of N-WASP, and its dia-binding region (DBR) to associate with the ankrin-rich repeats (ARR) of mDia2. **(b)** IRSp53 uses its IMD domain to bind and induce curvature of membranes and its SH3 domain to interact with the proline-rich region (PRR) of N-WASP and WAVE2, the PRR of Ena/VASP, and the proline-rich FH1 domain of mDia2. **(c)** DIP/WISH uses its SH3 domain to bind the PRR of N-WASP and an acidic (A-like) domain to bind Arp2/3 complex, leading to N-WASP-Arp2/3 activation, and its leucine-rich repeat (LRR) domain to directly inhibit FH2 domain of mDia2. (d) Spire uses its KIND domain to inhibit the FH2 domain of Cappuccino, while this same interaction enhances Spire activity.