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The WH2 Domain and Actin Nucleation – Necessary but Insufficient

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Abstract

Two types of sequences, proline-rich domains (PRDs) and the WASP-Homology 2 (WH2) domain, are found in most actin filament nucleation and elongation factors discovered thus far. PRDs serve as a platform for protein-protein interactions, often mediating the binding of profilin-actin. The WH2 domain is an abundant actin monomer-binding motif consisting of ~17-aa. It frequently occurs in tandem repeats, and functions in nucleation by recruiting actin subunits to form the polymerization nucleus. It is found in Spire, Cobl, Lmod, Arp2/3 complex activators (WASP, WHAMM, WAVE, etc.), the bacterial nucleators VopL/VopF and Sca2 and some formins. Yet, it is argued here that the WH2 domain plays only an auxiliary role in nucleation, always synergizing with other domains or proteins for this activity.

Opinion

Actin nucleators

Nucleation, i.e. the formation of a polymerization nucleus (or seed) consisting of two or more actin subunits, is the rate-limiting step during actin polymerization [1]. This biochemical property puts nucleation and the proteins that mediate this activity (nucleators) at the center of most actin assembly functions, by allowing cells to control polymerization in time and space and to specify the type of actin structures to be generated. For instance, cells generate branched actin networks in lamellipodia and parallel actin bundles in filopodia by engaging two different types of actin polymerization machineries, Arp2/3 complex and formins, respectively.

In accordance with their diverse subcellular localizations and different regulatory and nucleation mechanisms, actin filament nucleators are generally unrelated. Yet, they share one property – the ability to recruit two or more actin subunits to form a short-lived polymerization nucleus, which can either elongate to form a filament or disassemble. Most filament nucleators use WASP-Homology 2 (WH2) domain-related sequences for actin subunit recruitment (**Box 1**), and they typically also contain Pro-rich domains (**Box 2**).

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Numerous reviews address actin nucleation, and several are referenced here. Thus, the goal here is not to review actin nucleation, but to critically reevaluate the role of the WH2 domain in this activity by *a)* highlighting the widespread presence of the WH2 domain among filament nucleators, *b)* describing the basic principles that govern its activity, and *c)* developing the idea that the WH2 domain plays only an auxiliary role in nucleation, always synergizing with other domains or proteins for this activity.

The WH2 domain is the most abundant actin-binding motif among actin nucleators

Actin filament nucleators are generally unrelated, and use different actin-binding domains or combinations of domains for interaction with actin. Yet, WASP-Homology 2 (WH2) domains or WH2-domain-related sequences participate in actin-subunit recruitment in most actin nucleators [2-5], including nucleation-promoting factors (NPFs) of Arp2/3 complex and some formins (**Figure 1**). The omnipresence of the WH2 domain among filament nucleators is likely due to several factors, including its structural simplicity, short length, significant actin-binding affinity, and the fact that it binds actin at a site that overlaps only partially with inter-subunit contacts in the filament (**Box 1**, **Figure 2** and **Figure S1**). In a group of nucleators, known as tandem WH2-domain-based nucleators, the WH2 domain occurs in repeats of up to four domains (**Figures 1** and **2A**). These proteins include Spire, Cordon Bleu (Cobl), and the bacterial nucleators VopL/VopF and Sca2. When isolated from their native proteins, the WH2 repeats of some of these proteins display nucleation activity, leading to the initial idea that tandem WH2 domains were alone sufficient to drive nucleation and thus constituted a distinct subfamily of actin nucleators [4, 6-12]. However, evidence is emerging that the WH2 domain is insufficient to drive efficient nucleation, even when present in the form of tandem repeats, and other domains or proteins must also participate for optimal nucleation activity. Below, I discuss the experimental evidence supporting this conclusion for each nucleator subfamily.

Spire

Spire was the first protein shown to nucleate actin polymerization by a mechanism distinct than that of formins or Arp2/3 complex [6]. Spire contains a central repeat of four WH2 domains (**Figure 1** and **2A**), which accounts for the nucleation activity of the full-length protein, leading to the definition of a novel class of filament nucleators based on tandem WH2 domains. Importantly, linker-3 (between WH2 domains 3 and 4) was found to play a crucial role in Spire nucleation. Rotary-shadowed electron microscopy [6] and small angle x-ray scattering [11] suggest that when the linkers between WH2 domains are short as in Spire (13 to 15 amino acids), such repeats stabilize linear arrays of actin subunits along the long-pitch, two-start filament helix (**Figure S1**). However, this arrangement appears suboptimal for nucleation, presumably because the long-pitch helix of the double-stranded filament is not an ideal catalyzer of nucleation [13], and the flexible linkers between WH2 domains cannot properly align the actin subunits for nucleation. Indeed, it was immediately noted that the nucleation activity of Spire was much lower than that of Arp2/3 complex activated by WASP-family NPFs [6].

Coincidentally, the *spire* gene had been initially identified in a screen for female sterile mutants in *Drosophila melanogaster*, which also identified another gene, *cappuccino*, as

having a phenotype nearly identical to that of the *spire* mutant [14]. It was subsequently determined that the two gene products, Spire and the formin Cappuccino (**Figure 1**), interacted with each other [15, 16] and cooperated for the formation of an actin mesh during *Drosophila* oogenesis [17]. A similar interaction and functional cooperation during oocyte maturation was established between their mammalian orthologs, Spir1 and Spir2 and the formins Fmn1 and Fmn2 [16, 18]. The interaction proceeds through the formation of a 1:1 complex between the KIND domain of Spire and the C-terminal tail of the formins [19, 20]. The formation of this complex inhibits the nucleation and barbed end-binding activities of the formins, whereas the nucleation activity of Spire is significantly enhanced [16, 19]. Because Spire, which is itself monomeric, dimerizes upon binding to the formins, the cause of the increase in activity is believed to be duplication of the WH2 repeat, allowing for the recruitment of actin subunits along the two parallel strands of the two-start filament helix (**Figure 3**) [2, 16]. Consistent with this idea, forced dimerization of the Spire repeat of WH2 domains using the dimerization domain of another nucleator, VopL, leads to a dramatic increase in the nucleation activity [21]. It must be finally noted that while the majority view is that Spire's cellular activities depend on its ability to nucleate actin polymerization [2, 22], a different view is that Spire's main physiological activity is barbed end capping [23].

Cobl

Similar to Spire, Cobl contains a repeat of three WH2 domains, located at the C-terminus of the protein (**Figures 1 and 2A**). Cobl was found to nucleate actin polymerization, and this activity was mapped to the WH2 repeat (mouse Cobl residues 1127-1337) and shown to be important for its role in neuronal morphogenesis [9]. Cobl is enriched in the brain, where it plays an important role in dendritic arborization [9, 24, 25]. Cobl's nucleation activity has also been linked to primary cilia formation [26, 27] and microvillar assembly [28] and length regulation [29].

Different from Spire, Cobl's WH2 repeat presents a long linker of ~65-aa between the second and third WH2 domains (linker-2). Replacing this linker with a different sequence of similar length had nearly no effect on nucleation, whereas shortening the linker to 15-aa abolished the nucleation activity, leading to the initial proposal that Cobl stabilizes a short-pitch actin trimer during nucleation [9]. A subsequent study, however, found that the WH2 repeat of Cobl had no nucleation activity on its own, and instead inhibited actin assembly through monomer sequestration [30]. These authors identified a short lysine-rich sequence (K-region) immediately N-terminal to the WH2 domains that, together with the WH2 domains, was required for nucleation, albeit by itself this sequence did not bind actin. The K-region was also present in the construct used in the original study [9], although its importance was not immediately recognized. While the K-region along with the first WH2 domain was sufficient to nucleate polymerization, the nucleation activity increased incrementally with the addition of each WH2 domain, resulting in a ~10-fold higher activity when all three WH2 domains were present [30]. This result also appears to suggest that the long linker-2 plays a less critical role in nucleation than initially thought. At Cobl concentrations higher than 200 nM (corresponding approximately to three actin subunits per WH2 domain in polymerization assays with 2 μ M actin, 10% pyrene-labeled), other effects are observed following the initial nucleation phase, including monomer sequestration,

filament severing and depolymerization. However, actin is one of the most abundant proteins in nature whereas Cobl appears to be expressed at low levels (The Human Protein Atlas), suggesting that such high ratios of Cobl to actin are unlikely to occur under physiological conditions. It thus remains to be tested whether Cobl's primary physiological activity is nucleation [9, 24, 28, 29] or filament severing and monomer sequestration [30] (see Outstanding Questions).

While the K-region can turn the otherwise inactive WH2 repeat of Cobl into an actin nucleator, Cobl's overall nucleation activity is low, lower than that of Arp2/3 complex and possibly even lower than that of Spire under similar conditions [3]. The question arises: do other factors enhance Cobl's activity in cells? Remarkably, the cellular activities of Cobl depend on interactions with several cofactors, which are all expected to form dimers and would thus induce Cobl dimerization. This includes the BAR domain proteins syndapin, SNX9 and ASAP1 [24, 28, 29] as well as Abp1 [25], a protein featuring a predicted coiled coil domain in the middle of the sequence. All these proteins contain SH3 domains, which appear to interact with Cobl's PRDs. The available evidence is insufficient to propose a firm nucleation model for Cobl. However, it is tempting to propose that Cobl dimerization through interaction with any of these cofactors could increase its nucleation activity the way formins do for Spire (see Outstanding Questions). A hypothetical model is shown in **Figure 3**.

VopL/VopF

Vibrios are Gram-negative bacteria that cause illnesses ranging from mild cases of gastroenteritis and wound infection to life-threatening conditions such as septicemia [31]. Some *Vibrio* species produce a type III secretion system (T3SS) virulence factor that acts as a potent actin nucleator, including *V. parahaemolyticus*'s VopL [7] and *V. cholerae*'s VopF [32]. Through their ability to disrupt actin homeostasis, VopL/VopF are thought to contribute to host cell entry and infection.

The presence of three WH2 domains in VopL/VopF led to their initial identification as filament nucleators [7, 32]. However, analogous to Cobl, subsequent work showed that the WH2 domain repeat had no nucleation activity on its own, and that nucleation additionally required a unique ~240-aa VopL/VopF C-terminal domain (VCD) that mediates dimerization and pointed end binding [21, 33]. A recent crystal structure shows that VCD organizes three actin subunits at the pointed end into a filament-like arrangement, and suggests that the WH2 domains contribute to the nucleation activity by delivering actin subunits at the barbed end of the trimeric seed stabilized by VCD [34]. While the evidence is rather strong in support of this model [21, 33, 34] (**Figure 3**), a diametrically opposing view is that VopL/VopF's main functions are barbed-end uncapping, processive barbed end elongation and monomer sequestration [35, 36].

Sca2

Spotted fever group rickettsiae (SFGR) are tick-borne Gram-negative bacteria that cause diseases such as Rocky Mountain spotted fever and rickettsialpox [37]. Similar to *Listeria monocytogenes*, SFGR use an actin comet tail mechanism for motility. Yet, unlike *Listeria*

and other pathogens that use actin-driven motility, SFGR use two independent actin polymerization pathways [38]. Thus, early after infection motility is slow, driven by short and curved comet tails that are formed by RickA, an NPF expressed on the surface of the bacterium that recruits and activates the host cell Arp2/3 complex. Later during infection, motility is faster and directionally persistent, and the comet tails are long and straight, and are formed by Sca2, an autotransporter protein that accumulates at the bacterial pole.

Sca2 displays actin assembly properties that resemble those of eukaryotic formins; it nucleates unbranched actin filaments, processively associates with growing barbed ends, requires profilin-actin for efficient elongation, and competes with capping protein [10]. Sca2 has a central repeat of three WH2 domains (**Figure 1**), but this repeat is only partially responsible for actin assembly by Sca2. In what could be considered an evolutionary tour de force, Sca2 combines properties of both tandem WH2 domain-based nucleators and formins into a new fold, suitable for passage across the narrow pore formed by its translocator domain in the outer membrane of the bacterium [39]. Unlike formins, Sca2 is monomeric, but has N- and C-terminal repeat domains (NRD and CRD) that interact with each other for processive barbed-end elongation. The structure of NRD reveals a crescent-like fold consisting of a series of helix-loop-helix repeats, whose overall dimensions and shape resemble one of the subunits of the formin-homology 2 (FH2) dimer. CRD also contains helix-loop-helix repeats, and is predicted to have the same fold as NRD. In this way, NRD and CRD may form an FH2-like doughnut at the barbed end. In between the NRD and CRD, Sca2 contains two PRDs that mediate the recruitment of profilin-actin for elongation. The WH2 repeat, which is located in between the two PRDs, sequesters actin monomers when in isolation, analogous to Cobl's repeat [30]. However, disabling the WH2 domain through mutagenesis shows that within the full-length protein the WH2 repeat is essential for nucleation, although it does not participate in elongation [39]. Although the existing evidence allows to propose a tentative model of Sca2's nucleation and elongation mechanism (**Figure 3**), several features of this model remain untested and should inspire further studies on this protein (see Outstanding Questions).

Lmod

Four highly conserved Tropomodulin (Tmod) and three relatively less conserved Leiomodin (Lmod) isoforms are expressed in muscles and, to a lesser extent, other tissues [40]. While Tmods and Lmods are generally related, they have radically different activities: filament pointed end capping [40] and nucleation [41], respectively. Consistently, Tmods and Lmods do not compete with each other *in vitro* and display similar but distinct localization in sarcomeres. Thus, in cardiomyocytes, Tmod1 localizes exclusively to filament pointed ends near M-lines, whereas Lmod2 is enriched near M-lines but is also diffusely localized along the length of the thin filaments [42, 43]. Sequence differences along the polypeptide chains of Tmods and Lmods account for their different activities and localization. Tmods consist of alternating Tropomyosin (TM)- and actin-binding sites (TMBS1, ABS1, TMBS2, and ABS2) that mediate interactions with two TM and three actin molecules at the pointed end of the filament [44]. Lmods are distinguished from Tmods by the presence of a C-terminal extension featuring a PRD and a WH2 domain (**Figures 1 and 2A**), two elements found among most nucleators. Yet, this extension accounts only marginally for the different

activities of Tmods and Lmods. Indeed, Tmod fails to gain strong nucleation activity even after addition of the C-terminal extension of Lmod, whereas Lmod retains significant activity after removal of this extension [43]. Nucleation by Lmods is primarily the result of two major adaptations – the loss of pointed-end-capping elements present in Tmods, including ABS1, TMBS2, and the N-terminal portion of ABS2, and sequence variations within the otherwise highly conserved ABS2, allowing this domain in Lmod to recruit two or more actin subunits for nucleation (**Figure 4**). Thus, similar to the other nucleators described here, the WH2 domain-containing tail of Lmod plays an auxiliary role [43], possibly by helping to recruit the third actin subunit of the polymerization nucleus [45]. ABS2 binds at the interface between three actin subunits in the filament [43, 44], including most likely the subunit bound to the WH2 domain, which explains why this domain plays the most important role in nucleation by Lmod.

NPFs of Arp2/3 complex

The Arp2/3 complex is a ubiquitous assembly of seven proteins, including two actin related proteins (Arp2 and Arp3). The complex performs three major biochemical activities – filament nucleation, filament branching and pointed end capping of the branch – and participates in a myriad of cellular activities that require branched polymerization, including cell motility endo- and exocytosis and organelle trafficking [46]. The two Arps in the complex can be conceptually viewed as a polymerization nucleus. However, by itself the complex is inactive, because the Arps are splayed out in a conformation that is inconsistent with polymerization. The complex is activated by a family of NPFs that do three major things: a) they trigger a conformational change in the complex that repositions the Arps into a filament-like conformation, b) they contribute actin subunits for polymerization at the barbed end of the Arps, and c) they promote binding of the complex (and the newly formed filament branch) to the side of a preexisting (mother) filament (**Figure 5**).

NPFs share little in common, other than their C-terminal region comprising PRD, WH2 domain(s), Central (C) and Acidic (A) domains that bind, respectively, profilin-actin, actin, and Arp2/3 complex itself. This region is in itself sufficient to activate the complex *in vitro*. The other domains of NPFs are typically involved in regulation and/or localization, allowing for Arp2/3 complex to be exquisitely regulated in time and space. Moreover, most NPFs are large multi-protein complexes, and are themselves strictly regulated (for a recent review, see [46]).

It was previously thought that Arp2/3 complex was activated by a single NPF, but a series of studies have now shown that the complex is maximally activated by two NPFs [47-50]. Thus, like in other nucleators, the WH2 domains of NPFs play an auxiliary role in nucleation, by delivering actin subunits at the barbed end of the nucleus formed by Arp2 and Arp3 (**Figure 5**). Also analogous to other nucleators, NPF dimerization and/or oligomerization through interaction with BAR domain proteins, membranes, and other ‘clustering’ factors emerges as an important component of Arp2/3 complex-mediated nucleation [51].

Recently, a new class of NPFs, the WISH/DIP/SPIN90 family, was found to activate Arp2/3 complex to form linear (i.e. unbranched) actin networks [52]. These new NPFs do not appear

to bind actin monomers or filaments directly, and can thus activate Arp2/3 complex without the need for the mother filament.

Formins

Formins [53] are unique among filament nucleators in that they remain bound at the barbed end after nucleation, protecting this end from capping proteins and promoting processive filament elongation from profilin-actin. These properties allow formins to mediate the assembly of cytoskeletal structures consisting of unbranched filament bundles, such as filopodia, stress fibers, and the cytokinetic ring. Formins have been implicated in numerous physiological processes ranging from cell motility to tissue morphogenesis [53].

Formins vary in their domain architecture, but most contain formin-homology 1 (FH1) and 2 (FH2) domains lying adjacent to each other. The FH1 domain is a PRD that mediates the binding of profilin-actin. The FH2 domain forms a dimeric doughnut-like structure and mediates nucleation and barbed-end binding. Processive elongation requires both the FH1 and FH2 domains, and is accelerated by profilin-actin [54]. It was initially thought that formins relied solely on the FH2 domain for nucleation, despite the fact that for some formins this domain has nearly no nucleation activity on its own (e.g. DAAM1). Yet, a series of studies have now shown that sequences C-terminal to the FH2 domain strongly enhance nucleation, by either directly recruiting actin monomers or other proteins that synergize with formins for nucleation and other activities.

Thus, several formins, including INF2 [55], FMNL3 [56], mDia1, DAAM1, Bni1 and Bnr1 [57] contain WH2 or WH2-like domains near their C-termini that cooperate with the FH2 domain for actin assembly by contributing actin monomers during nucleation (**Figure 6**). For some of these formins, the actin monomer-binding site coincides (or partially overlaps) with the diaphanous autoregulatory domain (DAD) that participates in autoinhibitory interactions with the N-terminal diaphanous inhibitory domain (DID). For such formins, enhancement of the nucleation activity results from two separate effects: a) recruitment of actin monomers for nucleation, and b) the release of DID-DAD autoinhibitory interactions through competitive binding of actin to the DAD [57, 58].

In other formins, sequences C-terminal to the FH2 domain serve to recruit proteins that either contribute actin monomers for nucleation or take on the role of nucleation entirely (**Figure 6**), while allowing the formin to proceed with the elongation function. By analogy with Arp2/3 complex, such formin “helpers” have been named NPFs [53]. A well-characterized example is the synergistic interaction of the formin Cappuccino with Spire (and their mammalian orthologs Fmn1/Fmn2-Spir1/Spir2) described above [16, 18]. A similar cooperation exists between mDia1 and adenomatous polyposis coli (APC) [59]. The two proteins cooperate for filament assembly, with APC being mainly responsible for the nucleation step. Like Spire, APC can nucleate on its own, which depends on dimerization and the binding of two actin monomers per APC molecule [60]. Unlike Spire, however, the actin-binding sites of APC do not appear to correspond to WH2 domains, but the interaction is likely to proceed through a similar binding of an amphipathic helix to the target-binding cleft in actin, as observed with most actin interactions [61, 62]. After nucleation, APC and mDia1 dissociate, with APC remaining at the pointed end and mDia1 driving processive

barbed end elongation [59]. Another example of NPF-formin cooperation occurs between the yeast formins Bni1 and Bnr1 and the actin-binding protein Bud6, which increases the nucleation activity of the formins [63]. The C-terminal portion of Bud6 consists of a dimeric “core” region that binds to a region overlapping the DAD domain of Bni1 and Bnr1 [64] and a “flank” region that binds one actin monomer [65]. Because one Bud6 dimer binds to each of the tails of the formin dimer, Bud6 contributes a total of four actin monomers during nucleation. Although it could not be anticipated from sequence analysis, a recent crystal structure shows that the actin-binding site of Bud6 is partially related to the WH2 domain [65]. It consists of two helices; one mimicking the interaction of the helix of the WH2 domain with the target-binding cleft of actin, and the second one rising along the face of actin, analogous but not overlapping with the LKKV motif of the WH2 domain. It remains to be shown whether the interaction of APC with actin proceeds via a similar mechanism.

Other nucleators

Two nucleators not specifically discussed here are *Burkholderia* BimA [66] and *Chlamydia* TARP [67]. Different *Burkholderia* species produce different forms of the trimeric autotransporter protein BimA, involved in actin-based motility of the pathogens by mimicking different host actin polymerization mechanisms [66]. Thus, *B. thailandensis* BimA contains a WCA-like sequence and functions as an NPF, inducing filament nucleation and branching by activating the host cell Arp2/3 complex. In contrast, *B. mallei* and *B. pseudomallei* BimA resemble eukaryotic Ena/VASP in that they mediate processive barbed end elongation and compete with capping protein for binding to the barbed end, but contrary to Ena/VASP they also nucleate polymerization. While BimA proteins contain WH2-like domains, these sequences diverge quite significantly from the canonical WH2 domain, specifically lacking a proper “LKKV” motif, which is the most distinctive feature of the domain. The nucleation mechanism of *Chlamydia trachomatis* TARP is less well understood, but appears to involve actin binding through a short sequence that lacks clear resemblance to the WH2 domain and oligomerization *via* a PRD [67].

Concluding Remarks

The examples described here illustrate an emerging trend – actin nucleation almost invariably requires the presence of WH2 domain-related sequences, but this domain typically plays only an auxiliary role in this activity, synergizing with other domains or proteins for optimal nucleation activity. The WH2 domain, even when present in the form of tandem repeats, may lack the ability to properly position the actin subunits for nucleation, owing to the flexible nature of the linkers between WH2 domains. To generate a nucleation-competent arrangement of actin subunits in the polymerization nucleus, filament nucleators use other proteins or domains, specific to each nucleator. Experimental evidence for some of the ideas and models presented is still lacking, and should inspire further research in this area (see Outstanding Questions).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Box 1: The WH2 domain

WH2 domain-related sequences are found in most filament nucleators (**Figure 1**). As all the structures of WH2-actin complexes invariably show, the WH2 domain consists of 17-20 amino acids (**Figure 2**), folded as an N-terminal α -helix, which binds in the mostly hydrophobic target-binding cleft at the barbed end of the actin monomer, and an extended C-terminal portion that raises along the actin surface, roughly following the ridge between the outer (subdomains 1 and 2) and inner (subdomains 3 and 4) domains of the actin monomer [5, 68]. This binding site overlaps only partially with inter-subunits contacts in the filament [11, 12, 69] (see also Online Supplemental Information Figure S1), and makes binding of the WH2 domain sensitive to the nucleotide state of the actin monomer, showing a clear preference for polymerization-ready ATP-bound monomers [68, 70-72]. The extended C-terminal region comprises the so-called LKKT motif, a nomenclature resulting from the existing relationship between the WH2 domain and thymosin- β 4 [68, 73]. However, sequence analysis of a large number of WH2 domains shows that the actual consensus sequence in the WH2 domain is LKKV (**Figure 2**). While the α -helix and LKKV motif are the most distinctive features of the WH2 domain, neither is particularly well conserved, which, combined with its short length, make the WH2 domain difficult to identify from sequence alone. For instance, the WH2 domain is the main actin-binding motif among members of the Ena/VASP family of filament elongation factors, where it was initially called the G-actin-binding (GAB) domain (**Figure 2**). It was only with the subsequent biochemical and structural characterization of the GAB domain-actin interaction that the relationship with the WH2 domain became fully apparent [74, 75]. A similar situation occurred with several formins, where the presence of monomer-binding WH2-domain-related sequences was established only recently [55-57]. Despite its short length and variable sequence, most WH2 domains bind ATP-actin with low micromolar and, in some cases, nanomolar affinity [68, 70-72], making it an ideal monomer recruitment domain.

Box 2: Actin nucleators typically contain proline-rich domains (PRDs)

Proline-rich domains (PRDs) are abundant among actin assembly factors, including filament nucleation and elongation factors (**Figure 1**), but their precise role is not always understood. It is clear, however, that there is order within the apparent monotony of proline-rich sequences, which typically harbor specific protein-protein interaction motifs. Thus, PRDs can serve as a platform for the recruitment of profilin-actin, which accounts for the largest fraction of monomeric actin in cells [1]. This is because, opposite to its actin-binding site, profilin has a shallow cleft lined by aromatic residues that can specifically interact with at least eight amino acids, conforming to the sequence motifs PPPPPPPP that binds profilin bidirectionally [76] or PPPPPPLP that binds unidirectionally [75]. Shorter poly-Pro sequences also bind profilin, but with much lower affinity [77]. In this way, profilin serves as a bridge between actin monomers and filament assembly factors that harbor such sequence motifs. PRDs can also mediate interactions with ligands containing SH3 (binding motif: PxxP, where x is any amino acid) [78], EVH1 (binding motif: FPPPP) [79], and WW (binding motif: PPxY) [80] domains.

- WH2 domain-related sequences have been found in the majority of actin filament nucleation and elongation factors discovered thus far, including several formins.
- Tandem repeats of WH2 domains, found in proteins such as Spire, Cobl, VopL/VopF and Sca2, are no longer considered sufficient to drive efficient nucleation. These proteins cooperate with other proteins or present other domains necessary for efficient nucleation.
- It is now understood that optimal nucleation activity by several formins requires sequences C-terminal to the FH2 domain that either directly recruit actin monomers via WH2-like domains or bind to other proteins that contribute actin subunits during nucleation.
- Cooperation among different subfamilies of actin nucleators and dimerization are emerging as two widespread features in actin nucleation.

Outstanding Questions

Some of the ideas and models proposed here remain untested and should inspire further work, including:

- It remains to be demonstrated whether Cobl dimerization through interaction with dimeric cofactors such as syndapin, SNX9, ASAP1 and Abp1 increases its nucleation activity the way formins do for Spire.
- The nucleation mechanism proposed for Sca2 remains largely untested. Thus, it is unknown if CRD actually shares the fold of NRD, and whether the two together form a doughnut-like structure that binds the barbed end analogous to the formin FH2 domain. It is also unclear why the WH2 domains of Sca2 are spaced 40-50-aa apart, compared to 13-15-aa in Spire. Is this because of a folding requirement specific to autotransporter proteins?
- While most believe that VopL/VopF nucleate from the pointed end, followed by fast dissociation, at least one laboratory has proposed that these proteins bind at the barbed end and control filament elongation. Direct observation of VopL/VopF on actin filaments in the presence of known pointed-end- and barbed-end-binding proteins may help resolve this controversy.
- The interaction of APC with actin has not been structurally characterized. While *bona fide* WH2 domains are not readily detectable in APC, general principles described here would suggest that this interaction might proceed by a WH2-like mechanism.
- While *in vitro* studies of isolated WH2 domains or WH2 repeats, typically at high concentrations, have led some to suggest that some tandem WH2 domain nucleators (Spire, Cobl, VopL/VopF) display activities such as filament severing and barbed end capping, it remains to be demonstrated whether the full-length proteins exert any of these functions within a physiological context.

Figure 1.

Domain diagram of representative members of the four major nucleator subfamilies. The WH2 domain is shown in red and labeled as W. Proline-rich sequences are shown in cyan and labeled as PRD. Other domains specific to each protein include: KIND, kinase noncatalytic C-lobe domain; S, Spire box; FYVE, Fab1/YOTB/Vac1/EEA1 zinc-binding domain; K, basic K-region; SS, signal sequence; NRD, N-terminal repeat domain; CRD, C-terminal repeat domain; AC, autochaperone domain; TMBS1, tropomyosin-binding site 1; ABS2, actin-binding site 2; WH1, WASP-homology 1 domain; B, basic domain; CRIB, Cdc42/Rac interactive binding; C, central region; A, acidic region; GBD, GTPase-binding domain; DID, diaphanous inhibitory domain; DD, dimerization domain; FH2, formin-homology 2 domain; DAD, diaphanous autoregulatory domain. The UniProt accession codes of the proteins shown are: *Drosophila melanogaster* Spire, Q9U1K1-1; human Cobl, O75128-1; *Vibrio parahaemolyticus* VopL, Q87GE5; *Rickettsia conorii* Sca2, Q92JF7; human Lmod2, Q6P5Q4-1; human N-WASP, O00401; human WAVE2, Q9Y6W5-1; mouse WHAMM, Q571B6; *Rickettsia conorii* RickA, Q92H62; mouse FMNL3, Q6ZPF4; mouse mDia1, O08808; human INF2, Q27J81-1; and *Drosophila melanogaster* Cappuccino, Q24120.

Figure 2. Sequence and structure of the WH2 domain. **(A)** Alignments of the WH2 domains and WH2-related sequences of the proteins discussed here. Conservation scores for each amino acid were calculated based on a larger alignment of 100 representative sequences of WH2 domains from different proteins and species (not shown). Ten of the amino acids positions of the WH2 domain are conserved in more than 50% of the sequences (consensus 50%). The UniProt accession codes of the sequences shown are: human WASP, P42768; *Drosophila melanogaster* Spire, Q9U1K1-1; human Cobl, O75128-1; *Vibrio parahaemolyticus* VopL, Q87GE5; *Rickettsia conorii* Sca2, Q92JF7; human Lmod1, P29536; human Lmod2, Q6P5Q4-1; human N-WASP, O00401; human WAVE1, Q92558; human WAVE2, Q9Y6W5-1; human WHAMM, Q8TF30; *Rickettsia conorii* RickA, Q92H62; *Saccharomyces cerevisiae* LAS17, Q12446; human WIP, Q8TF74; human MIM, O43312; *Dictyostelium discoideum* actobindin, Q55DU1; *Schizosaccharomyces pombe* PAN1, Q10172; human Espin, B1AK53; human INF2, Q27J81-1; mouse mDia1, O08808; mouse FMNL3, Q6ZPF4; and human VASP, P50552. **(B)** Structure of the WH2 domain of WASP (the founding member of the WH2 domain family) bound to actin (PDB code: 2A3Z) [68]. The actin subdomains are labeled 1 to 4. **(C)** WH2 domain of human WASP, showing the side chains of the 10 residues that are conserved in more than 50% of the sequences, which most interact with actin.

Figure 3.

Hypothetical nucleation mechanisms of tandem WH2 domain-based nucleators. Proteins and domains are colored and labeled according to **Figure 1**. Note that for this group of nucleators, and specially Sca2 and Cobl, evidence is still lacking concerning the precise mechanisms of nucleation (see Outstanding Questions and main text). For Spire-Cappuccino [2], VopL/VopF [21, 33, 34] and Sca2 [39] similar mechanisms have been proposed, and are supported by structural and biochemical data. The mechanism proposed for Cobl is only supported by cellular data showing strict cooperation between Cobl and dimeric BAR domain proteins [24, 28, 29], and the widespread importance of dimerization (oligomerization) among filament nucleators. Except in the case of formins and Sca2, which function both as nucleation and elongation factors, these proteins are thought to be dissociate after nucleation, which is at least in part due to competition between binding of the WH2 domain and inter-subunit contacts in the filament (Figure S1). Filaments then elongate at barbed end from either monomeric actin or profilin-actin.

Figure 4.

Proposed nucleation mechanism of Lmod. Protein domains are colored and labeled according to **Figure 1**. Lmod contains two actin-binding sites (ABS2 and WH2 domain) and additionally interacts with Tropomyosin (TM) through TMBS1 [41, 43, 44]. ABS2 binds at the interface between three actin subunits of the filament. Lmod dissociates after nucleation, and is replaced at the pointed end by Tmod in muscle sarcomeres. Dissociation is possibly triggered by steric hindrance of the WH2 domain as well as the lack of capping elements present in Tmod, including ABS1, TMBS2, and the N-terminal portion of ABS2, which together contribute to the very high affinity of Tmod for pointed ends.

Figure 5.

Proposed nucleation mechanism of NPFs-Arp2/3 complex. Proteins and domains are colored and labeled according to **Figure 1**. The 7-subunit Arp2/3 complex contains two actin related subunits (Arp2 and Arp3). Interaction with two NPFs [47-50] repositions the Arps into a filament-like conformation and triggers binding on the side of the mother filament. Additionally, NPFs contribute actin subunits to the incipient branch. NPFs dissociated after nucleation, and the branch filament is free to grow from either monomeric actin or profilin-actin.

Figure 6.

Proposed nucleation mechanism of formins $\pm$ NPFs. Proteins and domains are colored and labeled according to **Figure 1**. Most formins contain formin-homology 1 (FH1) and 2 (FH2) domains. The FH1 domain is a PRD that mediates the binding of profilin-actin. The FH2 domain is responsible for nucleation and barbed-end binding. For several formins, sequences C-terminal to the FH2 domain enhance nucleation, by either directly recruiting actin monomers [55-58] (top model) or by recruiting other proteins that synergize with the formins during nucleation [16, 18, 59, 60, 63] (bottom model). Formins remain processively bound at the barbed end after nucleation, and accelerate barbed end elongation in a profilin-actin-dependent manner [54].