are required for cilia formation[16], pose challenges for defining the relationship between the cilium and PCP signalling.

Some studies support the view that the cilium directs Wnt signalling towards the PCP pathway and away from β-catenin activation[8,10], possibly through Dvl titration[10] (Fig. 1). Intuitively, this seems reasonable as the cilium is a postmitotic structure and PCP is often a differentiation feature, whereas β-catenin signalling is mostly mitogenic[1,2]. Interestingly, Katsanis and colleagues showed that the ability of Wnt5a, which activates a non-canonical pathway, to antagonize Wnt3a/β-catenin signalling is lost when *bbs4* is knocked down, a finding that seems to be consistent with such a 'cilium-switch' model. One may speculate that the cilium has been co-opted to integrate or balance β-catenin and PCP signalling during vertebrate evolution. However, it is not entirely clear whether the cilium enhances or suppresses PCP signalling, as both loss and gain of PCP signalling yield similar gross phenotypes[4]. It also remains possible that some ciliary proteins may be found to have positive effects on Wnt/β-catenin signalling.

As the loss of the cilium elevates Wnt/β-catenin signalling, why do mouse or fish embryos with abnormal ciliogenesis not exhibit overt patterning defects? Importantly, why are human ciliopathies not typically associated with tumorigenesis, as β-catenin signalling induces proliferation? The answer may lie in the observation that loss of cilia sensitizes cells to, but does not mimic, Wnt stimulation[8,9]. Although elevated β-catenin levels and nuclear accumulation are observed in Kif3a-deficient cells, β-catenin-dependent transcription remains low without Wnt[8,9]. This suggests that cilia loss does not result in constitutive β-catenin signalling, pointing to differences between the downstream effects of the high β-catenin levels observed after loss of cilia and the activated β-catenin signalling associated with, for example, the loss of APC tumour suppressor, which leads to tumorigenesis[2]. Another consideration is that the defective cilium impairs broad growth factor responsiveness[5,6], thereby preventing cell-fate change or transformation. Finally, one wonders whether elevated Wnt/β-catenin signalling underlies, at least in part, ciliopathogenesis. From mouse and fish models of PKD[10,13,17], it seems that deficiency of either Inv or Kif3a is indeed associated with elevated β-catenin signalling[10,13], and conversely, elevated β-catenin signalling following APC deletion causes PKD as well as renal tumorigenesis[17]. Overall, it is clear that understanding the connection between cilia and Wnt signalling will have broad implications for development and disease. From a signalling perspective, it seems likely that the list of proteins controlling Wnt/β-catenin and PCP signalling in vertebrates will grow as the functional ciliary proteome expands.

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Actin nucleation: bacteria get in-Spired

Margot E. Quinlan and Eugen Kerkhoff

Spir proteins nucleate actin polymerization by assembling a linear actin oligomer along a cluster of four actin-binding WH2 domains, and this process is enhanced by formins of the Cappuccino family. The discovery of Spir-like proteins in bacteria indicates that pathogens have adopted this mechanism to manipulate the host actin cytoskeleton.

The assembly of monomeric actin into filaments is a tightly regulated and essential cellular process. Spontaneous polymerization is inhibited both by the instability of small actin oligomers and by actin-monomer-binding proteins, such as profilin, which prevent the formation of such oligomers[1]. Consistent with the diversity of cellular actin structures, different mechanisms exist to generate (or nucleate) new actin filaments. Nucleation factors such as the Arp2/3 complex, formins and Spir all stabilize an actin oligomer to overcome the kinetic barrier to nucleation[1]. Their mechanisms are distinct and, accordingly, they are associated with different cytoskeletal structures. Recent studies have shed light on how Spir and one particular formin, Cappuccino, cooperate[2,3]. Moreover, the discovery of the bacterial nucleation factors VopF and VopL, which seem to use a Spir-like mechanism to nucleate actin, suggests that this pathway may have been adopted by pathogens[4,5].

A true nucleator must meet several criteria: first, it should accelerate *in vitro* actin polymerization independently of filament severing or increased elongation rate; second, a physiologically relevant nucleator should produce filaments that elongate from their barbed ends; third, a nucleator must recruit profilin-bound ATP–actin for filament assembly, either independently (as do formins) or with a cofactor (as

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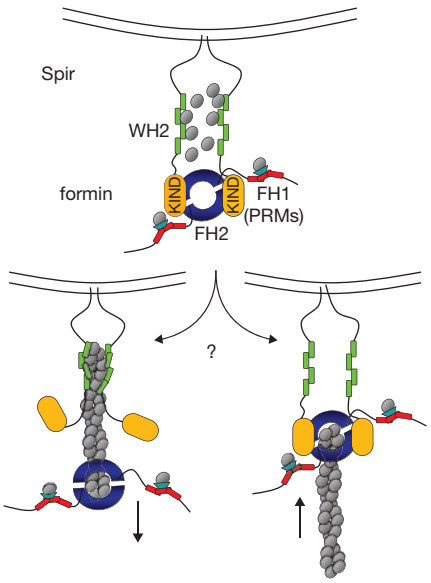

Figure 1 Model of Spir and Cappuccino cooperation in actin nucleation/polymerization. Spir and Cappuccino bind with a ratio of one Spir molecule per formin subunit. In addition, Spir proteins might dimerize and bind membrane with their C-terminal FYVE-like zinc fingers. These interactions arrange the WH2 clusters near each other, potentially creating a zone of high actin concentration and increasing the nucleation rate. A Spir-nucleated filament is arranged with its barbed end facing Cappucino. We speculate about two scenarios that follow nucleation: (**a**) If the KIND–FH2 interaction is released, a filament with both ends bound will continue to grow. (**b**) The initial nucleus could dissociate from Spir, but remain attached to Cappucino. If Spir is anchored to a membrane by its FYVE-like zinc finger, the filament might flip around. In this case, FH2-mediated elongation requires release from inhibition by the KIND domain, perhaps through a conformational change. Key structures are highlighted: WH2s (green); KIND (yellow); FH1 (PRMs, red); FH2 (blue); profilin (light blue); actin (grey).

with the Arp2/3 complex); fourth, a nucleator must be associated with an *in vivo* structure; finally, to understand the nucleation mechanism, a minimal domain, which contains actin binding sites and a means of coordinating a nucleus, must be identified.

Spir meets most of these criteria. It has been shown that it nucleates *de novo* filaments that elongate from their barbed ends[6]. The nucleation activity of Spir resides in a cluster of four actin-binding WASP homology 2 domains (WH2 A–D) separated by three conserved linker regions (L 1–3; ref. 6). Linker 3 overcomes the kinetic barrier of dimer formation by coordinating monomers in a structure that is believed to lie along one strand of the long-pitch actin helix[6]. Once a monomer is added to any of three potential cross-filament binding sites, polymerization proceeds. Notably, Spir lacks a proline-rich domain and does not efficiently nucleate profilin–actin[6]. This suggests that Spir requires a cofactor and both our recent findings [3] and those of St Johnston and colleagues[2] strongly suggest that Cappucino-family formins serve this function.

Formins are large modular proteins with two conserved formin-homology domains (FH1 and FH2) in their carboxyl termini[7]. The FH2 domain forms a dimeric ring that nucleates actin polymerization, probably by binding to two actin monomers[7,8]. After nucleation the FH2 dimer remains processively associated with elongating barbed ends of actin filaments, protecting the filament from being capped while allowing it to grow[7,8]. FH1 domains vary in length and composition. They are proline-rich and contain several potential profilin-binding sites[7]. Profilin can bind to an actin monomer and a proline-rich region simultaneously[9]. It has been proposed that actin bound to the FH1 domain through profilin can be rapidly transferred to the barbed end of a growing filament[10–12].

The first evidence of cooperation between Spir and Cappucino came from studies of *Drosophila melanogaster* genetics. *Drosophila spire* and *cappuccino* mutants were identified in the same genetic screen and have almost identical phenotypes[13]. A direct interaction of the Spir and Cappuccino proteins was first shown by a study of the Parkhurst group[14]. The authors described a novel function of Spir and Cappuccino, namely, the crosslinking of microtuble and actin filaments. On the basis of crosslinking experiments, they have proposed a model for Spir–Cappuccino cooperation during *Drosophila* oogenesis, which is independent of actin nucleation. The model, however, is in conflict with previous data and also with two recent studies[2,3]. Cooperation of Spir and Cappucino in actin nucleation was strongly supported by the recent findings of St Johnston and colleagues[2]. Analysis of *Drosophila spire* and *cappuccino* mutants showed that both proteins regulate a common actin structure — an isotropic mesh of actin filaments in the oocyte cytoplasm. Their findings that both proteins must cooperate to form a normal mesh led them to suggest that the proteins cooperate in actin nucleation. Recently, we confirmed that Spir and Cappucino proteins interact directly with each other, and showed that this interaction is conserved in mammals[3]. The interaction is mediated by the Spir kinase non-catalytic C-lobe domain (KIND) and the FH2 domain of Cappucino. The Spir–Cappucino interaction blocks actin nucleation through the formin and enhances nucleation by Spir. The FH2 dimer binds two KIND domains (Fig. 1). This stoichiometry brings eight WH2 domains in proximity to each other, explaining the observed enhancement of nucleation. It is possible that the Cappucino FH1 domain compensates for the lack of profilin binding by Spir. Nucleation by Spir results in a nascent filament with its barbed end pointing towards Cappucino. After nucleation by Spir, we propose two different possibilities (Fig. 1). The exact mechanism of the cooperation is unclear and requires further study.

The actin cytoskeleton is a major target for many pathogens. They rearrange actin to adhere to, enter, and spread from cell to cell. Bacteria have devised a number of strategies to manipulate and/or hijack the actin cytoskeleton[15]. One well-known phenomenon is to manipulate the Arp2/3 complex: either by recruiting its activators or mimicking the complex itself. Now proteins containing a Spir-like mechanism have been discovered in bacteria. These factors — VopF in *Vibrio cholerae* and VopL in *Vibrio parahaemolyticus*[4,5] — are important for virulence of these strains. In both cases, they alter the actin cytoskeleton *in vivo* and accelerate polymerization *in vitro* using WH2 domains. VopL does not alter elongation kinetics or have severing activity[4]. Although untested, the same property is likely to be true for VopF based on sequence similarity[5]. Experiments with cytochalasin D suggest that VopF nucleates filaments that grow from the barbed end[5] and experiments with capping protein clearly demonstrate that VopL-nucleated filaments elongate from the barbed end[4]. Both proteins contain three WH2 domains (Fig. 2a, b). As with Spir, these domains are essential to the nucleation mechanism[4–6], although how VopF and VopL coordinate actin monomers to form a nucleus is unknown. Neither protein has a sequence similar to Linker 3 of Spir, yet they are potent nucleators[4–6].

In addition to the WH2 domains, both VopF and VopL contain three copies of a proline-rich motif (PRM) (Fig. 2a, c)[4,5]. The PRMs may contribute to nucleation by binding to profilin–actin. This activity must be tested, however, because although the PRMs are well conserved, they are short compared with the minimum predicted profilin-binding site[12]. In the case of

VopF, when the first two PRMs are deleted the steady-state filamentous actin concentration is significantly reduced, suggesting that this construct sequesters monomers[5]. Regulation of sequestration would be a new and surprising activity for these domains. VopL also binds to the sides of actin filaments[4]; however, we do not know if this activity is linked to, or independent of, nucleation activity. There may be other domains that contribute to the nucleation activity. Nonetheless, a minimal region necessary for nucleation must be identified in order to establish the mechanism of polymerization and understand the contributions of other actin-binding activities such as sequestration and filament-binding.

Both VopF and VopL are associated with actin-based structures *in vivo*. VopL induces abundant stress fibres and binds along their lengths, possibly through its side-binding activity[4]. VopF expression induces actin-rich protrusions[5]. Curiously, VopF is found at the tips of these protrusions, apparently associated with the barbed end of actin filaments. This observation is reminiscent of formin FH2 domains, which bind the barbed end of actin filaments and allow them to elongate from this end. Although VopF does not contain FH2 domains, it is structurally possible for the WH2 domains to associate with the exposed barbed end of actin filaments: WH2 domains in several different proteins have been shown to bind between subdomains 1 and 3 of actin monomers, which are exposed at the barbed end of actin filaments[9]. This observation raises the possibility that the bacterial WH2 clusters could bind to a growing barbed end, although it must be emphasized that, unlike formins, VopL does not protect the barbed end from capping protein[4,7].

In eukaryotes, Cordon-Bleu, a second WH2-based nucleator, was recently described, [16]. It is expressed most highly in the brain and regulates the induction of neurites and neurite branching. In transient expression studies in COS-7 cells it accumulates in areas of ruffling, where it colocalizes with F-actin. Cordon-Bleu contains three WH2 domains and multiple PRMs including one between the second and third WH2 domains, similar to that found in the bacterial nucleators (Fig. 2a)[4,5,16].

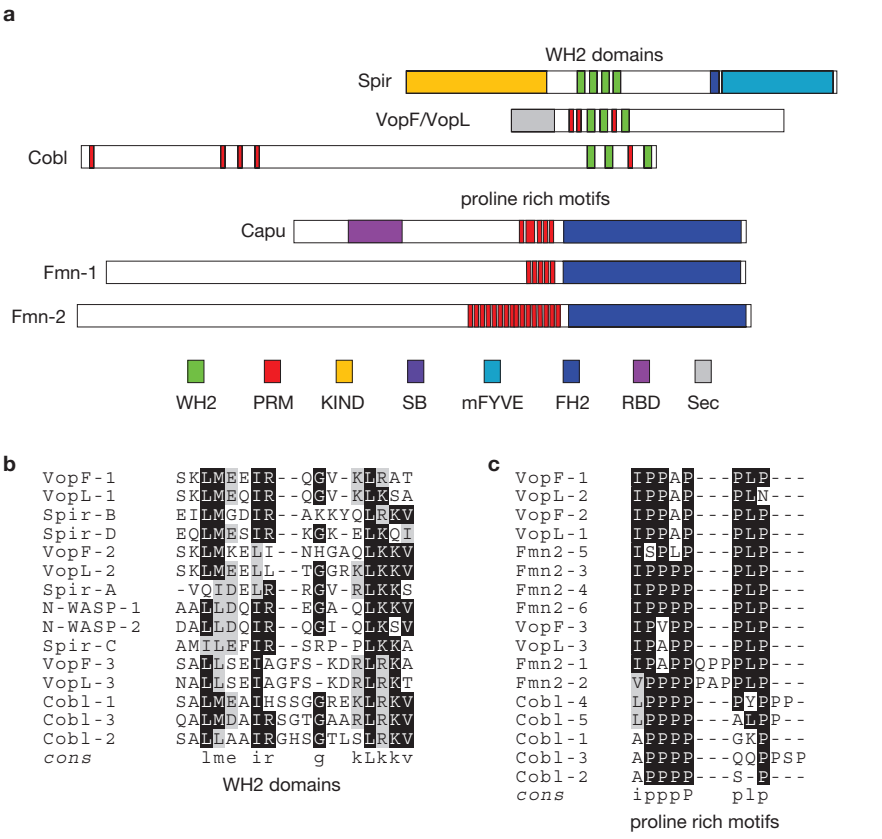

Figure 2 Comparison of WH2-based nucleators and formin structures. (**a**) Domain organization of Cappuccino-family formins, Spir, VopF, VopL and Cobl. WH2 (actin monomer binding domains); PRM (proline rich motifs); KIND (kinase non-catalytic C-lobe domain); SB (Spir box); mFYVE (modified FYVE domain); FH2 (forming-homology 2 domain); RBD (putative Rho-binding domain); Sec (secretion/translocation domain). (**b**, **c**) Clustal W was used to align WH2 domains (**b**) and PRMs (**c**) of these proteins. The bottom line of each alignment is a consensus sequence based on homology greater than 50%.

Together these findings establish actin nucleation by multiple WH2 domains as a general mechansim. The discovery that pathogens manipulate the actin cytoskeleton has provided many tools and great insight into the mechanism of nucleation by the Arp2/3 complex. By studying this bacterial virulence system, we also gain a new opportunity to learn about Spir-like mechanisms of actin nucleation.

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